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IN VITRO EFFECACY OF METHANOLIC EXTRACTS OF *VERNONIA AMYGDALINA*, *CROTON MACROSTACHYUS*, *RICINUS COMMUNIS* AND PETROLEUM ETHER EXTRACT OF *MILLETTIA FERRUGINEA* AGAINST *BOVICOLA OVIS* AND *RHIPICEPHALUS* (*BOOPHILUS*) *DECOLORATUS*

MSc Thesis

BY

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BISHOFTU, ETHIOPIA

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A thesis submitted to College of Veterinary Medicine and Agriculture of Addis Ababa University in partial of the requirement for the Degree of Master of Science in Tropical Veterinary Parasitology

By

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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the thesis prepared by **Askale Gizaw** titled "***In vitro* Efficacy of methanolic extracts of *Vernonia amygdalina, Croton macrostachyus, Ricinus communis* and petroleum ether extract of *Millettia ferruginea* against *Bovicola ovis* and *Rhipicephalus (Boophilus) decoloratus*"** and recommend that it be accepted as fulfilling the thesis requirement for the degree of Masters of Science in **Tropical Veterinary Parasitology.**

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First, I declare that this thesis is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for MSc degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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TABLE OF CONTENTS

Table of contents (continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF ANNEXS

LIST OF ABBREVIATIONS

AAU	Addis Ababa University
ANOVA	Analysis of Variance
CSA	Central Statistics Authority
CVMA	College of Veterinary Medicine and Agriculture
ESGPIP	Ethiopia Sheep and Goat Productivity Improvement Program
HSD	Honestly Significant Difference
SE	Standard error
Spp.	Species
SPSS	Statistical Package for the Social Sciences
WHO	World Health Organization

ABSTRACT

In vitro loucicidal and accaricidal efficacy evaluation of Vernonia amygdalina, Croton macrostachyus, Ricinus communis and Millettia ferruginea against Bovicola ovis and Rhipicephalus decoloratus of cattle were carried out from January 2014 to April 2015. Crude methanol extracts of Vernonia amygdalina, Croton macrostachyus, Ricinus communis leaf and Petroleum ether extract of Millettia ferruginea seed oil were prepared for in vitro test at different time intervals. The four selected medicinal plants, at concentrations of 200, 100, 50, 25, 12.5 and 6.25mg/ml and a commercially used acaricide (0.1% diazinon), were examined using in vitro adult immersion test. In vitro loucicidal test showed all concentration of *Millettia ferruginea oil (µl/ml), ≥25m g /ml concentration of Croton macrostachyus and 200 and 100mg/ml concentration of Ricinus communis had pronounced loucidal activity at 24hr of exposure. 200mg/ml concentration of Vernonia amygdalina and ≤6.25mg/ml concentration of Croton macr ostachyus had moderate loucicidal activity at 24hr of exposure. Moreover, the 200 and 100µl/ml concentration of Millettia ferruginea seed oil and 200mg/ml concentration of Ricinus communis leaf crude extract had high accaricidal activity (90-100%) against Rhipicephalus decoloratus while crude methanolic extracts of Croton macrostachyus and Vernonia amygdalina and even the positive control (diazinon) showed low acaricidal activity (<35%) against the tick species. All plants had significantly (P< 0.05) higher activity against lice than Rhipicephalus decoloratus ticks except Millettia ferruginea at (200 and 100µl/ml) and Ricinus comm unis (at 200 mg/ml) which showed both accaricidal and loucicidal activity. Therefore the present study concluded that Croton macrostachyus, Ricinus communis and Millet tia ferruginea against Bovicola ovis whereas Ricinus communis and Millettia ferruginea against Rhipicephalus decoloratus could be used as potential alternative in the discovery of guide compounds that substitute commercially available acaricides. In addition, the low activity of diazinon on ticks compared to our plant extracts deserves further attention.*

***Key Words**: Acaricide, Bovicola ovis, in vitro, loucicide, medicinal plants, Rhipicephalus decoloratus*

1. INTRODUCTION

Loss of productivity in livestock sector is of multifactorial nature. Diseases are one of them which are significantly reducing the quantity and quality of animal products and by products. Parasitic diseases (endoparasites and ectoparasites) are global problems and considered a major obstacle in the health and product performance of animals (Furman and Loomis, 1984). Ectoparasites are very common and widely distributed in all agro ecological zones in Ethiopia (Kumsa *et al*., 2012). Ectoparasites pose serious economic losses to the farmer, the tanning industry and the country as a whole (Berhanu *et al.,* 2011).

Among ectoparasites, ticks and lice are very important and harmful parasites. Lice are one of the most common and economically important ectoparasites of domestic animals including sheep. Both biting and suckling lice affect sheep (Radostits *et al*., 1994). The most common lice affecting sheep are *Bovicola ovis* (Cotter, 2013). The sheep biting louse, *Bovicola ovis*, is an economically important ectoparasite found throughout most sheep raising areas of the world (Levot, 1995). *B. ovis* cause significant economic loss by irritating sheep and reducing wool quantity and quality (James, 2008). *Rh. decoloratus* is one of the most important cattle ticks in Ethiopia for its parasitic effect. Heavy infestations of *Rh. decoloratus* are likely to cause damage to hides and to reduce the rate of growth of cattle. Moreover, *Rh. decoloratus* transmit s the protozoan *Babesia bigemina*, *Anaplasma marginale* and *Borrelia theileri* (Walke r *et al*., 2007). Generally infestation with these ectoparasitic skin diseases is responsible for blood loss and irritation which results in downgrading and rejection of skins, poor growth, decreased production and reproduction and mortality.

As a result control strategies are implement to reduce disease and limit losses in the animal husbandry industry to or below acceptable economic damage thresholds (Plant and Lewis, 2011). Highly potent chemical insecticides such as organophosphate compounds are routinely and extensively used interventions in ectoparasite control of livestock. Although these are effectively used as chemical acaricides for livestock, drug residues that can build up through extensive and long term use pose an environm ental hazard and can lead to increased resistance in target species (Currie *et al*., 2004)

. Moreover, presence of chemical residues in meat, milk and the environment has also prompted interest in finding new alternatives. In addition to these their accessibility and affordability to the poor farmers makes them less preferable compared to other alternatives such as medicinal plants (Robert *et al*., 2010).

The majority of farmers and pastoralists in the developing countries rely on traditional health care practices to keep their livestock healthy. These indigenous practices include the use of medicinal plants or ethno-veterinary medicine (EVM) (Kaaya, 2003; Matlebyane *et al*., 2010). The application of botanicals to livestock to control ectoparasites of veterinary importance is widespread in the developing countries (Robert *et al*., 2010). In contrast, to chemical acaricides, botanical acaricides have many advantageous features of being degraded in the environment, do not remain in livestock, are not as prone to resistance, and are relatively safe for humans, animals, the environment (Alawa *et al.,* 2003). They have also greater accessibility with lower costs and apparent effectiveness (Mwale *et al*., 2005). However, limited research work are conducted in Ethiopia to exploit this potential.

In Ethiopia research works have shown 100% accaricidal efficacy of *Millettia ferruginea* on *Amblyomma variegatum* ticks (Kumer *et al*., 2013). In addition among nine potential plants mentioned by respondents in north Gondar that could be used to kill or repel ticks, *Millettia ferruginea* (birbira) was in the second rank (Melaku, 2013). But there is no study which shows the effect of *Millettia ferruginea* plant on *B.ovis* of sheep and *Rh*. *decoloratus* of cattle. In addition studies on the effect of *V. amygdalina* on tick, mites and mosquitoes, *C. macrostachyus* on mosquitoes and *R. communis* on tick, mosquitoes, and bedbugs are survey for their traditional uses (Karunamoorthi and Hailu, 2014). In botanical survey conducted in Akaki District, Eastern Shewa, Ethiopia *C. macrostachyus,* is taken as the dominant medicinal plant used by healers to control insects (Bekele *et al*., 2012). However, loucicidal and accaricidal activity of *V. amygdalina, C. macrostachyus, R. communis* and *M. ferruginea* scientifically were not evaluated. This indicates the need for the scientific evaluation of loucicidal and accaricidal efficacy of *V. amygdalina*, *C. macrostachyus*, *R. communis* and *M. ferruginea* plants against different species of lice and ticks. Therefore the general objective of this study was to evaluate efficacy of selected medicinal plants against known species of lice and ticks. The specific objectives were:

- To evaluate loucicidal efficacy of crude methanolic extract of *Vernonia amygdalina*, *Croton macrostachyus*, *Ricinus communis* and petroleum ether extract of *Millettia ferruginea* seed oil against *Bovicola ovis*.
- To evaluate acaricidal efficacy of crude methanolic extract of *Vernonia amygd alina*, *Croton macrostachyus*, *Ricinus communis* and petroleum ether extract of *Millettia ferruginea* seed oil against *Rhipicephalus decoloratus*
- To compare efficacy of the selected medicinal plants on *Bovicola ovis* and *Rhipicephalus decoloratus*

2. LITERATURE REVIEW

2.1. Ectoparasites

Ectoparasites are organisms which spend all or part of their life cycles on the external of another organism, the host, and in the process extract nutriment from it for survival. The presence of external parasites on the host is termed as infestation. The host provides a number of important resources for the parasite, most vitally, the host serve as a source of food, which may be blood, lymph, tear or sweat or the debris of the skin, hair or feather and the environment on which ectoparasites live, generating warmth, moisture and within the skin or hair; a degree of protection from the external environment. The host may also provide transportation from place to place for the, a site at which to mate and, in many cases, the means of transmission from host to hos t. The association between arthropod ectoparasite and vertebrate hosts may take on variety of forms. In some cases the parasite may be totally dependent on the host, alternatively, the parasite may feed, or live only occasionally on the host, without being dependent on it (Wall and Shearer, 2001).

Ectoparasites feeding on host cause direct damage to skin and other subcutaneous tiss ue, inflammation and significant blood loss. This activity is usually associated with pruritis, erythema, excoriation, papules, lichenification, scale and crusting and self trauma. Wounds may be subject to secondary infestation or bacterial infection. The salivary and faecal antigens produced by ectoparasites as they feed can stimulate immune responses, in some individuals leading to hypersensitivity (Van den Broek *et al*., 2003). Importantly, some ectoparasites also act as vectors of protozoa, bacteria, viruses, cestodes and nematodes. The behavior of ectoparasites also may cause harm indirectly, causing disturbance, increasing levels of behavior such as rubbing, and leading to reduced time spent grazing or ruminating and, in some cases, to self-wounding (Berriatu *et al*., 1999). Lice and tick are among the ectoparasites leading impact directly and indirectly on livestock.

2.1.1. Lice Infestation

The lice belong to the Phylum Arthropoda, Class insecta, order Phthiraptera which is divided into four suborders; Anoplura, Amblycera, Ischnocera and Rhynchophthirina and family Trichodectidae. Amblycera and Ischnocera are known as chewing lice while Anoplura are described as sucking lice. Rhynchophthirina is a very small sub order that include just two species, one of which is a parasite of elephants and the other warthogs (Wall and Shearer, 2001).

They are small insects, about 0.5-8 mm in length, dorsoventrally flattened, wingless and possess stout legs and claws for clinging tightly to fur, hair and feathers. They feed on epidermal tissue debris, parts of feathers, sebaceous secretions and blood (Wall and Shearer, 2001). Biting lice graze on epidermal tissue, hair and other organic waste. They cause intense itching by their action. Sucking lice have a narrow head with mouthparts adapted for penetrating the skin of the host and sucking blood (ESGPIP, 2010).

Both biting and sucking lice affect small ruminants (Radostits *et al*., 1994). The most common lice affecting sheep are *Bovicola ovis* (Cotter, 2013). The sheep body louse (*Bovicola ovis*, formerly called *Damalinia ovis*) is a species found in genus *Bovicola* which is a chewing louse affecting sheep. *Bovicola ovis* is a pale yellow insect 1.5 to 2 mm long with brown transverse stripes on the abdomen and a broad, red-brown head (James, 2013). The sheep chewing lice, have a rounded head with a pair of three segmented antennae (Wall and Shearer, 2001). Males are smaller than females and have more pointed abdomens (James, 2013).

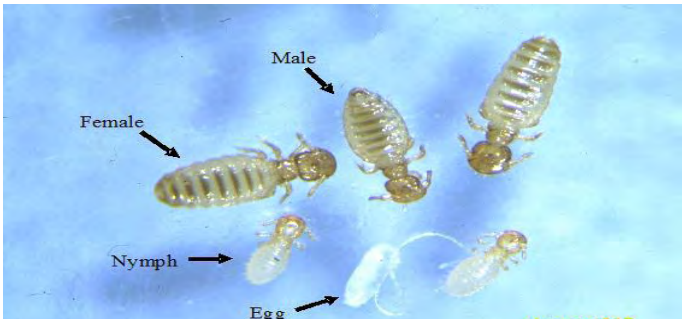

Figure 1: Sheep lice (*B. ovis*)
Source: (James, 2013)

B. ovis has typical life cycle. The female deposits about two eggs, attached to the wool or hair next to the skin by a viscid substance, every three days. The egg hatch in 9 - 10 days, and the nymph matures in about 21 days (Bay and Harris, 1988). Lice are obligate parasites, that is they cannot complete their life cycle away from their host. However, survival away from sheep for up to 29 days has been recorded by (Crawford *et al*., 2001).

Most species of louse are highly host specific and many species specialize in infesting only one part of their host body (Wall and Shearer, 2001) and transfer to new hosts is by body contact, particularly under condition of close confinement (Peter, 1995). Transmission from flock to flock is usually accomplished by introduction of infested animals to healthy flock (James, 2013). In addition inert objects such as blankets, grooming tools and harness may remain infective for several days. Sheep may become infested with foot lice from the pasture (Radostits *et al*., 1994). The rate at which transfer occurs will depend on the wool length, amount of close contact between sheep, time and strength of the stimulus for lice to move to the fleece surface, weather condition, density of lice on infested sheep and chemical treatment (James, 2013). To allow them survive as permanent ectoparasites, lice show a number of adaptations which enable them to maintain a life of intimate contact with their hosts (Wall and Shearer, 2001).

Lice respond to warmth, humidity and chemical odors. Many receptors are located on the antennae but heat and humidity receptors are located over the entire body. Solar radiation, rainfall, temperature, shearing are factors affecting louse numbers (James, 2013). Most lice populations on animals vary seasonally, depending on the condition of the host. Lice populations on animals are greater during the rainy months (Hailu, 2010). Lice have a tightly defined band of humidity and temperature preference and respond to humidity and temperature gradients by showing increased rates of turning in favorable microclimates which tend to keep them in favorable areas (Wall and Shearer, 2001). When temperature is cooler than optimum, eggs do not develop while hotter temperature prevent egg laying and kill the lice (Radostits *et al*., 1994). In addition, they usually move away from direct light towards dark objects (Wall and Shearer, 2001). In the flock, there are often carrier animals that stay heavily infested all year round (Urquhart *et al*., 1996). Animals under stress usually support larger lice

populations than found under normal conditions (ESGPIP, 2010). Lice infestation was reported to be higher in debilitated animals that suffer from malnutrition and intestinal parasitism (Pugh, 2002). Sheep susceptibility to lice is affected by breed, differences among sheep within breed, age and Sheep health and nutrition (James, 2013).

The common sites of infestation for *Bovicola ovis* were the skin of shoulder, neck, rump, flank, belly and back (Chanie, 2011). Infestations with *B. ovis* occur over all areas of the body but the upper sides of the animal are favored. This species move rapidly over the wool fiber but is usually found near the skin (Bay and Harris, 1988).

Figure 2: Sheep heavily infested with *Bovicola ovis* and eggs (nits) glued to the wool source : (Chanie *et al*., 2010)

The effect of lice is usually a function of their density (Wall and Shearer, 2001). All species cause irritation of the skin whist feeding and stimulate scratching, rubbing, and licking leading to restlessness, damage to the fleece and skin and animals exhibit reduced weight gain and loss in production. The saliva and feces of the lice contains substances, which are capable of causing allergies, giving rise to severe irritations, followed by the skin thickening and sometimes self trauma (Peter, 1995). This is usually shown by the animal rubbing itself against objects. General unthriftyness, matted, dull fleece with tufts of wool may indicate lice infestation (Holdsworth *et al*., 2006).

The sheep biting louse, *Bovicola ovis*, is an economically important ectoparasite found throughout most sheep-raising areas of the world (Levot, 1995). Being highly active, *B ovis*, is usually considered to be most pathogenic in sheep and it can cause great

irritation so that the sheep are restless and have their grazing interrupted. Exuded serum from the bite wounds cause wool matting. Rubbing leads to wool loss. Wound may attract blowflies (Wall and Shearer, 2001). Like keds, *B. ovis* is also associated with the development of cockle (Heath *et al*., 1996). Cockle is an inflammatory response of the skin to the presence of lice and their saliva. This is seen after the wool or hair has been removed from the skin. Animals in poor body condition are likely to be seriously affected (ESGPIP, 2010).

2.1.2. Tick infestations

Ticks are bloodsucking ectoparasites that can parasitize animals of different groups such as mammals, birds, reptiles, amphibians (Keirans and Durden, 2005; Anderson and Magnarelli, 2008). Ticks are animals belonging to the phylum Arthropoda (Anderson and Magnarelli, 2008). There are three families of ticks: the Argasidae, the Ixodidae and the Nuttalliellidae (Mehlhorn and Armstrong, 2010). The family, Ixodidae contains the important genera *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes* and *Rhipicephalus*. Also formerly of the genus *Boophilus*, are now classified as a sub-genus within the genus *Rhipicephalus* (Taylor, 2007). Family Argasidae contain the three veterinary important genera, *Argas*, *Ornithodoros*, and *Otobius* (Urquhart *et al*., 1996). Families Nuttalliellidae comprises a single species, *Nuttalliella namaqua* (Guglielmone *et al.,* 2010).

Ticks that are considered to be most important to domestic animals' health in Africa comprise about seven genera and forty species. Among these tick genera, the main ticks found in Ethiopia are *Ambylomma*, *Boophilus*, *Heamaphysalis*, *Haylomma* and Rhipicephalus (Minjauw and McLeod, 2003). Among these, *A. varigatum* and *B. decoloratus* are most important and widely distributed (Abebaw, 2004).

Rhipicephalus decoloratus is the commonest, most widespread and frequent of the one-host cattle ticks in Africa. *Rhipicephalus decoloratus* is known as the blue tick because of the color of engorged females. This species and the others in the *Boophilus* sub-genus within the genus *Rhipicephalus*. The character states that have been used for this sub-genus are separate from the rest of the genus *Rhipicephalus.* Cattle are the main host of *Rh. decoloratus* but it also feeds on horses, donkeys, sheep, goats and

wild ungulates. Cattle are probably the only maintenance host for this tick and infestations of other hosts will only occur when a population of ticks is maintained by cattle. The preferred feeding sites of all stages on cattle are, in order of preference: back, upper legs, neck, shoulders, dewlap and belly (Walker *et al*., 2007).

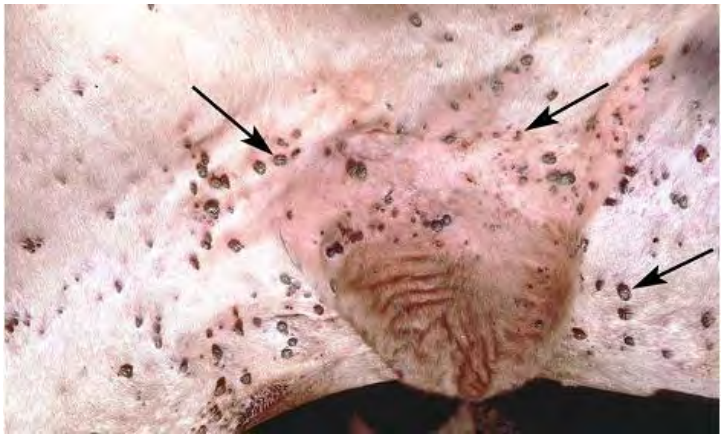

Figure 3 : *Rhipicephalus decoloratus* on belly
source : (Latif and Walker, 2004)

Rh. decoloratus is a one-host tick with a monotropic type of behaviour. The engorged females lay 1000 to 2500 eggs about one week after detaching from the host. The eggs hatch in 3 to 6 weeks and the larvae ascend the vegetation and wait there for a host. The larvae attach, engorge and moult to nymphs on the host after a week. The nymphs attach on the same host, engorge and moult to adults after a week. Finally the adults attach on the same host, partially engorge, mate and the females fully engorge and drop off after a week. The three stages spend a total of about three weeks on the same host and the life cycle, including the non-parasitic phase, can be completed in approximately two months (Walker *et al*., 2007).

Ticks attach predominantly on areas with thin skin, and survive particularly in anatomical regions which are non-accessible to grooming and licking; the proportion of ticks on various areas of the body surface therefore varies widely according to the tick species and stage. It is important to know the tick attachment sites for two purposes: to visually monitor tick infestations in a farm, and to focus acaricides applications and manual tick removal exclusively on specific body regions, thus reducing the quantity of acaricides and labor time and increasing the effectiveness of

these two control options. Different tick species have different locations of attachment (Mac Leod *et al.*, 1977). The location can indicate the type of tick. e.g *Boophilus microplus* Ear, limbs, dewlaps, abdomen and chest, *Rh. decoloratus*, Abdomen, limbs, dewlap and groin (ESGPIP, 2010).

The economically most important ixodid ticks of livestock in tropical regions belong to the genera of *Hyalomma*, *Boophilus*, *Amblyomma* and *Rhipicephalus* (Frans, 2000). In many areas of the world, tick-induced productivity and mortality losses inflict large costs on beef and dairy industries, and the problem remains especially severe in Africa. Ticks are responsible for severe economic losses both through direct effect of blood sucking and toxins (FAO,1998). These parasites generate direct effects in cattle in terms of milk production and reduce weight gain (L'Hostis and Seegers, 2002; Peter *et al*., 2005). Ticks are attached to the body for a blood meal and may cause irritation and serious physical damages to livestock including "tick worry", irritation, unrest, and weight loss due to massive infestation of ticks, direct injury to hides due to tick bites, and loss of blood due to the feeding of ticks (Drummond, 1983). Heavy infestations of *Rh*. *decoloratus* are likely to cause damage to hides and to reduce the rate of growth of cattle (Walker *et al*., 2007). Bekele (2002) estimated an annual loss of US$500 000 from hide and skin downgrading from ticks, and approximately 65.5% of major defects of hides in eastern Ethiopia are from ticks.

Ticks indirectly affect their host by transmitting a greater variety of pathogenic micro organism than other arthropod vector group while their blood meal (Jongejan and Uilenberg, 2004). *Rh. decoloratus* transmits the protozoan *Babesia bigemina*, causing bovine babesiosis (redwater) in cattle. The *Babesia* is transmitted only by the nymph and adult after it has passed transovarially from the previous generation of ticks. Once established in the tick host, *Babesia. bigemina* can be transmitted by many successive generations without their acquiring new infections. This tick transmits the bacteria *Anaplasma marginale,* the cause of bovine anaplasmosis (gall sickness) and *Borrelia theileri*, the cause of spirochaetosis in cattle, sheep, goats and horses (Walker *et al*., 2007).

2.2. Lice and Tick Control

Lice are permanent ectoparasites; consequently, their control is much easier than control of other temporary ectoparasites (Mitra *et al*., 2011). There are various methods of lice control measures implemented by farmers in developing countries. Most commonly used are commercial and ethno-veterinary remedies which are discussed below. Control of sheep lice depends almost exclusively on the application of chemical pediculicides and conventional treatments aim to deliver active concentrations of insecticide to all sites on the sheep where lice survive (Lund *et al*., 2000). Veterinary ectoparasiticides are designed to control a range of parasites in sheep, including ticks, lice, keds, blow-flies, head-flies and scab-mites. These can cause substantial financial losses and welfare concerns if left untreated (Levot and Sales, 2008).

Pyrethroids-cypermethrin, deltamethrin, alphamethrin, Organophosphates (diazinon) Benzoylphenyl urea, diflubenzuron, Ivermectin (injetting fluid), Magnesium fluorosili cate/sulphur approved for organics and Spinosad - approved for organics Imidacloprid are Chemical options for sheep lice control (Joshua *et al*., 2010). Generally, ectoparasiticides are applied to sheep topically as a dip, pour-on or spray (jetting), injections or impregnated ear-tags (Wall, 2007).

Despite this and the fact that, in many countries, sheep flocks are subjected to annual or biannual dipping and other insecticide applications, lice are still a problem in many flocks throughout the world. Owners blame neighbors or new sheep as the main reason for lice infestations in their flocks. However, investigations in Australia have shown that the main reason for infestations is the failure to eradicate the resident lice populations in the flock (Plant, 1993) because of the ineffective application of proper chemicals to the skin of the sheep. In addition, heavy use of chemicals, particularly with long wool treatments, can leave undesirable chemical residues in the wool (Morcombe *et al*., 1999), contribute to the development of resistance in lice populations (Levot and Sales, 2008) and pose occupational health and safety and environmental risks during application (Murray *et al*., 1992).

As *B. ovis* is a chewing louse and not blood feeding, systemic chemicals are not effective and treatment is usually by immersion dipping, high volume spraying or through the use of high concentration formulations that diffuse through the wool grease in the fleece and on the skin. Difficulty in completely covering the sheep and contacting all lice, particularly when sheep have not been recently shorn, often compromises treatment effectiveness (Lund *et al*., 2000).

Farmers mostly rely on the use of chemical acaricides and repellents to limit production losses. In order to reduce contact between ticks and vertebrate hosts, chemical repellents such as DEET and permethrin are extensively used (Faulde *et al*., 2003). Acaricides typically are highly lethal to ticks, and field applications generally are quite effective in reducing tick numbers (Sonenshine, 1993; Stafford and Kitron, 2002). Jernigan *et al*. (2000) demonstrated the acaricidal efficacy of Selamectin against experimentally induced ticks (*Rhipicephalus sanguineus* and *Dermacentor variabilis*) infestation on dogs. Witchey-Lakshman (1999) outlined the various class of etoparasiticide: Organophosphate (diazinon, fampur, phosmet, dichlorvos), synthetic pyrethroids (resmethrin, deltamethrin, permethrin), carbamates (carbofuran, propoxur), growth regulator (fenoxycarb, methroprene), amitraz, fipronil and methandiol that are currently being used for tick control. Although clearly effective at reducing transmission of tick-borne pathogens to livestock, repeated heavy applications of pesticides to hosts can cause considerable mortality in non-target arthropods through environmental contamination (Gassner *et al*., 1997).

2.3. Challenges and Alternatives to Lice and Ticks Control

The effective control of sheep lice with conventional pesticides can be hampered in some cases by the dense water repellent fleeces of sheep preventing contact between lice and the pesticide. This has prompted some interesting research into the use of entomopathogenic nematodes. These are motile parasites able to actively seek out insect hosts such as lice in the fleece of sheep. A study by James *et al* (2010) 0investigated whether the nematodes, *Steinernema carpocapsae*, *Steinernema riobrave*, *Steinernema feltiae* and *Heterorhabditis bacteriophora* were able to infect and kill *Bovicola ovis*. All were shown to infect and kill lice in Petri dish assays at 30^{o}C. It was concluded that *Steinernema riobrave* may likely be the most effective

against *Bovicola ovis* when applied to live sheep due to its greater tolerance to high temperatures and it foraging strategy.

A range of plant extracts or derivatives have been tested against lice. With sheep lice, Heath *et al*. (1995) found that dipping in a neem formulation gave a reduction in louse score of 85% to 100%. Guerinni (2000) reduced lice by 98 to 100% by spraying sheep with neem. Dimri and Sharma (2003) reported that dusting sheep with 25% neem powder and 75% sulphur removed 51% of lice. James *et al*. (2010) showed that a range of plant extracts including essential oils from eucalyptus, lemon scented myrtle, and tea tree and various neem extracts all had effect against sheep lice in laboratory studies.

Tick resistance to acaricides is on the rise due especially to increased frequency in the application of acaricides (Jonsson *et al*., 2000). For instance, *R. microplus* has developed resistance to synthetic pyrethroids and amitraz (Jonsson *et al*, 2000), amitraz, chlorfenvinphos and cypermethrin against *Boophilus decoloratus* (Mekonnen *et al.,* 2002). Environmental pollution is a serious problem posed by the use of synthetic acaricides in tick control. Chemical compound such as endosulfan and endosulfan sulphate are toxic and bioaccumulate in nature (Bhattacarya *et al*., 2003). Organophosphate accumulation in fatty tissue of mammals can lead to poisoning in man (Karalliede *et al*., 2003).

Ethno-veterinary medicine is very important in Africa and other developing countries since a greater proportion of livestock farmers are small-scale and most of these are in rural areas where cultural practices are still preserved (Madge, 1998). Plant extract preparations are developed by famers rather than scientists due to lack of finance to purchase synthetic acaricides which force them to depend on traditional methods of tick control. Furthermore, products from plant such as neem (*Azadirachta indica*) are easily biodegradable (Liang *et al*., 2003).

Several plants and herbs have been shown to possess anti-tick insecticidal, growth inhibiting, antimolting and repellent activities. A number of reports are available on the effect of different extracts of plant material on tick species. Preliminary results obtained by Indian workers (Ghosh *et al*., 2007) with alcoholic extracts of *Annona*

squamosa and *Azadirachta indica* against different life stages of *Hyalomma* and *Boophilus* are highly encouraging. Recently, Nana *et al*. (2010) demonstrated the attraction of *Rh. pulchellus* and *Rh. appendiculatus* to leaf extracts of *Calpurnia aurea* Benth (Fabaceae).

2.4. Study Plants in Ethno Veterinary Medicine

Ethno veterinary is a branch of science which deals with the study of traditional knowledge, methods, skills and practices used for treating various ailments of animals (McCorkle, 1986). According to the World Health Organization, a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi synthesis. Such a plant will have its parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition and therefore contains chemical components that are medically active. These non-nutrient plant chemical compounds or bioactive components are often referred to as phytochemicals or phytoconstituents and are responsible for protecting the plant against microbial infections or infestations by pests (Liu, 2004; Nweze *et al*., 2004; Doughari *et al*., 2009).

2.4.1. Vernonia amygdalina

V. amygdalina occurs naturally along rivers and lakes, in forests margins, woodland and grassland up to 2800 m altitude, in regions where mean annual rainfall is 750-2000mm (Ofori *et al*., 2013). *V. amygdalina* can be commonly found along drainage lines and in natural forests or at home and commercial plantations (Alem and Woldemariam, 2009). It requires full sunlight and prefers humid environment. It grows on all soil types but prefers humus-rich soils (Ofori *et al*., 2013).

According to (Erasto *et al*., 2006), the botanical overview of *Vernonia amygdalina* belongs to the family Asteraceae. The leaves are dark green coloured with a characteristic odour and a bitter taste (Bosch *et al*, 2005). Flower heads thistle like, small, creamy white, 10 mm long, grouped in dense heads, axillary and terminal, forming large flat clusters, 15 cm in diameter, sweetly scented (Ofori *et al*., 2013).

Figure 4: *V. amygdalina* (girawa)

source : (Ucheck , 2004)

The variety of secondary metabolites extracted from *V. amygdalina,* explains well the diversity of the biological activities of this plant extract. Leaf extract of *V. amygdalina* was found to contain reducing sugar, polyphenolics, terpenoids, saponins, alkaloids, cardiac glycosides, steroids or triterpenes, anthraquinone and coumarins without cyanogenic glycoside (Ayoola *et al*., 2008). However, only tannins, glycosides and saponins without flavanoids could be obtained from its root and stem bark extracts (Nduagu *et al*., 2008). All of these phytochemicals contributed to anticorrosion (Odiongenyi *et al.*, 2009) and antifungal effect (Nduagu *et al*., 2008) of *V. amygdalina* while its bitter taste was reported to be due to the presence of antinutritional factors such as alkaloids, saponins, tannins and glycosides (Arhoghro *et al*., 2009). Phenolic compounds identified in *V. amygdalina* can be grouped into flavonoids, tannins (Salawu and Akindahunsi, 2007).

In Ethiopia the plant is used in cleaning the containers used for fermentation purpose. Due to its bitterness, it also can be used as a bittering agent, a hop substitute and for the control of microbial contamination in beer brewing without affecting the quality of malt. In Ethiopia, it is used to make honey wine called Teij (Eleyinmi *et al*., 2004; Kasolo and Temu, 2008). The leaves are used for human consumption and washed before eating to get rid of the bitter taste. They are used as vegetable and stimulate the digestive system (Ofori *et al*., 2013). *V. amygdalina* with just a little amount of

processing can be classified as healthy food because it promotes the healthy development of the body (Iwu, 2002). Besides, this plant has also been widely used as fuel wood, stakes, fodder, construction poles, fencing of agroforestry buffer zone and as ingredient for compost (Eleyinmi *et al*., 2004; Kasolo and Temu, 2008). Decoction-s for the leaves of *V. amygdalina* are commonly used in traditional medicine to treat fever, malaria, diarrhoea, dysentery, hepatitis and cough, and as a laxative and fertility inducer (Farombi, 2003).

Vernonia amygdalina, also known as ‘‘African bitter leaf’’, is a plant vegetable used for both food and traditional treatment of diseases (Farombi & Owoeye, 2011). All parts of the plant are pharmacologically useful (Hamoiona and Saffaf, 1994). The usage of *V. amygdalina* as medicinal herb started when zoo pharmacologists found that sick chimpanzees with empty stomach sucked pith and juice from the unsavoury *Vernonia* plant stalk (which was not their common diet) for self-deparasitization, enhanced body fitness, increased strength or appetite and reduced constipation or diarrhoea especially during rainy season (Huffman *et al.,* 1997).

The ethanol, petroleum ether, dichloromethane, ethyl acetate, acetone-water and isoamyl alcohol extracts of *V. amygdalina*, showed antimalarial activity against *Plasmodium falciparum in vitro* (Madureira *et al*., 2002;Tona *et al.,* 2004). The methanolic and aqueous leaves extract also possessed antiamoebic activity at 100 to 500 µg/ml with 20 to 80% inhibition against *Entamoeba histolytica* after 3 to 4 days incubation (Otshudi and Foriers, 2000; Moundipa *et al*., 2005). Five consecutive days injection of 1mg/kg petroleum ether and ethanolic leaf extracts *V. amygdalina* possessed antischistosomiasis effect of parasite load) on mice infected with *Schistosoma mansoni* type cercariae (Ogboli *et al*., 2000). Methanol extract of *V. amygdalina* leaf induced 50% of mortality on *Spodoptera litura* through deterrent of feeding activity at 2 mg/ml and induced 50% of mortality on *Culex pipiense* pallense at 10 µg/ml (Ohigashi *et al*., 1991). One to five grams of *V. amygdalina* dry leaf powder was able to control the growth of *S. zeamais* and *Sitophilus oryzae* on stored maize grains and rice (Law-Ogbomo and Enobakhare, 2007).

Ethanol extract of the fruit (which is rarely found on most of the *V. amygdalina* shrub) possessed antiviral effect on polio virus (Vlietinck *et al*., 1995). Methanol extract of

V. amygdalina was reported to show strong antifungal activity on *Pseudoperonospora cubensis* and mild activity on *Rhizoctonia solani* (Ohigashi *et al*., 1991). Histopathological examination showed that as low as 15% of extract could even completely revert liver change to normal in the treated animals (Arhoghro *et al*., 2009). *V. amygdalina* was the most popular antidiabetic traditional herbal remedy in Nigeria (Gbolade, 2009).

2.4.2. Croton macrostachyus

The genus Croton belongs to the family Euphorbiaceae (which commonly known as the 'spurge' family) (Negash, 2010). It is known as Bisana' (in Amharic) (Karunamoorthi and Ilango, 2010). and consists of approximately 1300 species of trees, shrubs and herbs distributed in tropical and subtropical regions of the world (Salatino *et al*., 2007). Croton *macrostachyus* which is called 'rush foil' or 'broad-leaved croton is a multipurpose, medium sized, drought-deciduous pioneer tree. It is a tall tree found in tropical regions of Africa (Bum *et al*., 2012).

Elsewhere in Africa, *C. macrostachyus* has been reported to occur in Angola, Burundi, Cameroon, Central Africa, Ghana, Guinea, Ivory Coast, Kenya, Malawi, Mozambique, Nigeria, Rwanda, Sudan, Tanzania, Uganda, Zaire, and Zambia (Friis, 1992). It is native to Eritrea, Ethiopia, Kenya, Nigeria, Tanzania and Uganda (Kapingu *et al*.,2000; Orwa *et al*., 2009). In Ethiopia, *C. macrostachyus* occurs in regions between 1300 and 2500 m a.s.l with annual rainfall ranging between 750 and 2000 mm. Croton macrostachyus is commonly found on forest edges along rivers, around lakes, woodlands, wooded grasslands and along roadsides (Negash, 2010).

The tree is quite persistent, regenerating large numbers of coppices or shoots, even when it is repeatedly lopped or degraded. Provided that environmental and soil conditions are favorable, *C. macrostachyus* does establish well and can grow quite fast on reasonably good and well-drained soils, but prefers red or loam soils to verti soils. The latter soils are known for their shrink-swell properties (during the dry and wet seasons, respectively), and for getting waterlogged during the rainy season (Negash, 2010).

Croton macrostachyus (Euphorbiaceae) is a large tree with cylindrical trunk. The stem is more or less pyramidal in shape with widespread branches. The stem is gray clear, smooth and fissure with age. Leaves are almost as heart-shaped large that long, they have 10 to 15 cm of length, they are flexible, green or brunette according to the season and present some prominent ribs. Flowers are regrouped in inflorescence on stems of about 25 cm of long. They are visible but their life span is very short. They are colour creamy and slightly fragrant yellow. Fruits are regrouped along an axis (Stuart and Graham, 1973).

Figure 5: *Croton macrostachyus* ('bisana ')

source : (Yaw, 2012)

Previous phytochemical studies on the genus *Croton* resulted in the isolation of secondary metabolites such as alkaloids, terpenoids, flavonoids and other phenolic compounds. Amongst them, diterpenoids are the most widespread in this genus (Salatino *et al*., 2007). The seeds also contain several saponins and a resin, which is said to be more toxic to insects than rotenone. The stem bark and twigs contain lupeol, betulin and several fatty acids. The fruits contain crotepoxide, a cyclohexane diepoxide, which inhibits certain tumours in animal models (Tene *et al*., 2009)..

Croton macrostachyus has several other important uses, e.g. to control soil erosion, as a shade tree and for its green manure and fodder, which deserve additional tests in the

field. Ethnobotanical/pharmacological studies revealed that *Croton macrostachyus* has a wide range of activities. In Ethiopia, it is used for the treatment of malaria by the Shinasha, Agew-awi and Amhara people (Gidey *et al*., 2007). The plant has shown a promising antimicrobial (Kalayou *et al*., 2012), antidiabetic (Kapingu *et al*., 2000). The ethyl acetate extract of the stem bark (Mathieu *et al*., 2009) as well as the methanol and dichloromethane extracts of the leaves and stem bark of the plant have been demonstrated to possess significant antibacterial and antifungal activities (Taniguchi and Kubo, 1993). An in vitro study of the fruit extract indicated that the plant had a good antiplasmodial activity (Sorsa, 1992). Moreover, the methanol leaf extract of Croton macrostachyus exhibited larvicidal activity against late third instar larvae of *Anopheles arabiensis* Patton, a potent malaria vector in Ethiopia (Karunamoorthi *et al*., 2010). A number of plant families are known to produce alkaloids, phenolic and oils which have been used for insect control since a long time. They were called as insect killers and were used by Romans and Chineses (Heintz and Downum, 1987). Plant, *Croton macrostachyus* is well known for their medicinal and insects/mosquitoes repellent properties among the rural residents of Ethiopia (Karunamoorthi and Ilango, 2010).

2.4.3. Ricinus communis

Ricinus communis (commonly known as castor bean) belongs to family Euphorbiaceae (Ilavarasan *et al*., 2006). Castor plant is a tough annual that may grow up to 6 to 15 feet in one season when full sunlight, heat and adequate moisture conditions are available. It prefers hotter areas with temperature range of 20-26ºC with frost-free winters. It may live for many years and become quite woody and tree-like (Khafagi, 2007).

Leaves are simple and alternate and can grow very large; from 15 to 30 inches wide. The green to reddish leaves are lopsidedly peltate with the petiole attaching to the interior of the blade above the center point. Each leaf has 5 to 11 major veins radiating outward into narrow lobes with jagged margins. Flowers appear in summer and fall on tall spikes up to 18 inches long that grow out of the top of the stems. The fruit is a ½- to 1-inch diameter, spiny capsule that turns from yellow to blue-green and

then to brown as it matures. Each capsule houses three small, poisonous seeds that resemble dog ticks (Melissa *et al*., 2004).

Figure 6: *Ricinus communis* ('gulo')

source: (Melissa *et al*., 2004).

Ricinus communis is a plant commonly found in both the tropical and temperate climates of the world (Raoof and Yasmeen, 2006). Castor plant prefers hotter areas with temperature range of 20-26ºC with frost-free winters. It may live for many years and become quite woody and tree-like (Khafagi, 2007).

The seeds of *R. communis*, is biochemically composed of various macromolecules: the fat which is about 15% to 25% consists of about 40-53% of fixed oil comprising glycosides of ricinoleic, isoricinoleic, stearic and dihydroxy stearic acids (Lin and Areinas, 2007). Seeds contain three toxic proteins Ricin A, B and C and one ricinus agglutinin (Murthy *et al*., 2003). Leaves contains Disaccharide glycoside rutin, gentistic acid, quercetin, and gallic acid are determined in the dried leaves of *R. communis* (Laureano *et al*., 2009). Flavonoids (Chen *et al*., 2008) and tannins (Khafagy *et al*.,1979) have been isolated from the leaves. The pericarp of the fruits of *R. communis* contain alkaloid, ricinine (Ferraz *et al.*, 1999).

Ricinus communis is one of the medicinally important oil seed crop. The various solvent extractions prepared from the different parts of the plant have been reported to

possess medicinal properties hepatoprotective (Sabina *et al*., 2009), anti-diabetic (Shokeen *et al*., 2008), anti-fertility (Sandhyakumari *et al*., 2003), antimicrobial (Oyewole *et al*., 2010), analgesic (Williamson, 2002), antihistaminic and anti-inflammatory (Lomash *et al*., 2010), Hypoglycemic, Laxative (Kensa *et al.,* 2011), antifungal (Shariff *et al*., 2006), antiviral and cytotoxicity (Sokmen, 2001), and insecticidal (Tounou *et al*., 2011). *R. communis* seed extracts provided an excellent potential against the *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes albopictus* mosquitoes vector (Mandal, 2010). Extracts from leaves and seed of castor bean *Ricinus communis* have been used successfully in the management of curculionids of agricultural importance (Tinzaara *et al.,* 2006).

2.4.4. Millettia ferruginea

Millettia ferruginea is well known groforestry species only found in Ethiopia (Loha *et al*., 2008). It is belonging to the family Fabaceae (Leguminosae) (Thulin, 1983). The tree is commonly known as 'Berebera' (in Amharic), Sotallo, Kotalu, Sari, Yego (in Afan Oromo), Enghediksho (in Sidama), Zaghia (in Wolaita), Dhadhato (in Gedoffa) languages (Getahun, 1976). Flowering period usually extends from the end of February to the end of March. Its fruits are normally abundant from early June to end of December. Mostly from January to February, seeds are usually released by mechanical or explosive mechanisms from the pod to the ground (Andualem & Gessesse, 2010).

Most tree lengths ranged from 25 to 30 meter. Leaves are compound with the number of leaflets**.** In most trees, the flour is large with mostly violent on the stalks and golden-brown in colour. The mature pod is flat and pale brown in colour. The shape of the seeds is semi round and nearly golden-brown in colour (Andualem & Gessesse, 2010).

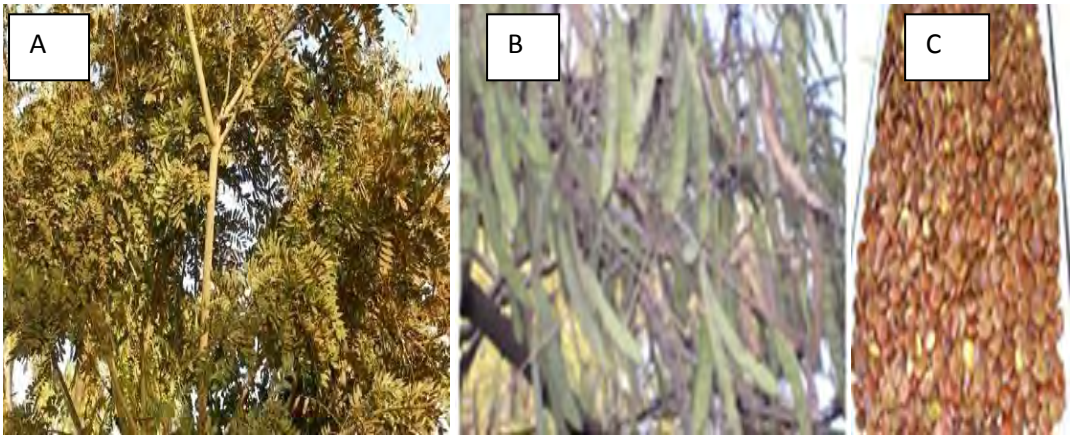

Figure 7: *Millettia ferruginea* tree ('birbira') (A), pod of *M. ferruginea* (B) and *M. ferruginea* seeds (C).

Source: Andualem & Gessesse, 2013, 2014).

The potential growing area of *Millettia ferruginea* ranges from 1000 to 2500m above sea level (Azene, 2007). That means most parts of Kola and the whole woinadega of Ethiopia are potential areas for *Millettia ferruginea* growth. Therefore, the natural habitat of *Millettia ferruginea* is rather diverse as it has advanced mechanism of minimizing water loss which enabled it to utilize water in a very conservative manner (Gindaba *et al*., 2004). It maintained higher tissue water status under mild, moderate and severe water stresses than the other species due to its ability to reorient its leaves and leaflets during midday, thus avoiding direct solar irradiance. *Millettia ferruginea is* commonly grown in Tigray, Gondar, Gojam, Shewa, Welega, Harerege, Bale, Ilubabor, Kefa and Sidamo (Thulin, 1989).

The birbira seed a potential of high oil and protein content to satisfy calorie and protein demand of the populations (Andualem and Gessesse, 2014). Oil is clearer brown yellow in colour and less viscous (Catarelli *et al*., 1993). In addition to its high protein content, birbira seed contains a high concentration of minerals, especially phosphorus, potassium, magnesium sodium and calcium (Andualem & Gessesse, 2010). It has a potential to supply sufficient amount of minerals for consumers and microbial media for microorganisms (Andualem and Gessesse, 2014). Rotenone is one of the dominant compounds found in the seed, stem and bark of *M. ferruginea* (Jembere *et al*., 2007). A variety of chemical compounds, notably rotenoids and isoflavones have been isolated from the bark, pods, seeds and root bark of *M.*

ferruginea (Dagne *et al*., 1990). The amount of rotenone of the seed was 0.701 ± 0.02%. It can be used as insect pesticide if production and application method is developed. Glutamic acid was the most predominant amino acid followed by aspartic acid, leucine, and lysine. The values of amino acids showed that cysteine and methionine were in the lowest levels (Andualem and Gessesse, 2014).

M. ferruginea is a multi-purpose tree and highly valued by farmers. *Millettia ferruginea* in agroforestry systems such as for erosion control (Tadesse *et al*., 2000; Azene, 2007), home gardens as shade/cover crop for coffee and enset or false banana (*Ensete ventricosum*) particularly in South and South-West Ethiopia (Andualem and Gessesse, 2013). The leaves of *M. ferruginea* can be used as fodder for ruminants. In addition, its wood can be used as firewood, as local building material, to make tool handles and household utensils, for fencing (Azene, 2007) and keeping traditional bee hives. Its flowers have good apicultural value as bee forage (Fichtl and Adi, 1994). Birbira seed extract is traditionally used as a herbal fish poison in Ethiopia, where mature pods and seeds are ground to fine powder and spread over the water surface for stunning fish (Karunamoorthi *et al*., 2009). The plant is traditionally used to treat skin infection (Mesfin *et al*., 2009) and for dressing 'mujele'fleas (Tunga) an infection caused by an insect present in the soil (Teklelehaymanot and Giday, 2007).

The efficacy of *M. ferruginea* has been tested against many insect pests. A laboratory study showed that water extracts of *M. ferruginea* seed powder at different concentration levels (10–40% w/v) caused 93–100% mortality against the different castes of adult *Macrotermes termites* at all concentration levels (Getahun and Jembere, 2006). Birbira seed water extract also caused 45–60% mortality on the sorghum chaffer Pachnoda interrupta (Oliver) within 24–48 h, which was found to be significantly higher than the mortality caused by the standard insecticide, carbaryl, applied at the recommended rate (1.5 kg/400 l of water) (Jembere *et al*., 2006). Eyob *et al*. (2010) reported that *M. ferruginea* caused a significantly higher level of mortality on the enset root mealybug (Cataenococcus ensete Williams and Matile-Ferrero) than Schinus molle L., *Melia azedarach* L. and *Phytolacca dodecandra* L. Damte and Chichaybelu (2002) also tested the toxicity of *Millettia* seed against the Adzuki bean beetle Callosobruchus chinensis, which gave complete protection of stored chickpea for 6 months in the laboratory. The seed powder from *M. ferruginea*

also caused 100% mortality in the bean weevil Zabrotes subfasciatus (Boheman) (Jembere *et al*., 2007).

George (1980) reported that rotenoids have been used as insecticides, when they were applied to plants to control leaf-eating caterpillars. Thus, rotenone, a non-specific botanical insecticide with some acaricidal properties, is not only used in home gardens for insect control, but also used for lice and tick control on pets and for fish eradications as part of water body management (Jembere, 2002; Hien *et al*., 2003). It is also one of the dominant compounds found in Millettia seeds (Dagne and Bekele, 1990; Jembere *et al*., 2007) that might possess insecticidal properties against the stemborer, although the other compounds could be equally important. Jembere *et al.* (2006) reported that the water extract of *M. ferruginea* seeds is the most active among the different extracts. In accaricidal efficacy evaluation of millettia ferruginea, 100% mortality of *Amblyomma variegatum* was observed with 20, 40, 60, 80 and 100 % seed oil after 12, 9, 6, 3 and 1.5h respectively (Kumar *et al*., 2013).

2.5. Plant Extraction

Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The products so obtained from plants are relatively impure liquids, semisolids or powders (Handa *et al*., 2008). A typical extraction process may contain steps of collection and authentication of plant material & drying, size reduction, extraction, filtration, concentration, drying & reconstitution (Handa *et al*., 2008). Quality of an extract is influenced by several factors such as, plant parts used, solvent used for extraction, extraction procedure, and plant material: solvent ratio etc. (Pandey and Tripathi, 2014). The choice will also depend on the targeted compounds to be extracted (Pandey and Tripathi, 2014). For selection of solvents *'like dissolves like'* principle is applicable. Thus polar solvents will extract out polar substances and non-polar material will be extracted out by non-polar solvents (Huie, 2002).

Table 1: Solvents used for active component extraction

Water	Ethanol	Methanol	Chloroform	Ether	Acetone
Anthocyanins	Tannins	Anthocyanins	Terpenoids	Terpenoids	Phenol
Starches	Polyphenols	Terpenoids	Flavonoids	Coumarins	Flavonols
Tannins	Polyacetyle	Saponins		Fatty acids	
Saponins	nes	Tannins			
Terpenoids	Flavonols	Xanthoxyllines			
Polypeptides	Terpenoids	Totarol			
Lectins	Sterols	Quassinoids			
	Alkaloids	Lactones			
		Flavones			
		Phenones			
		Polyphenols			

Source: (Pandey and Tripathi, 2014)

2.5.1. Plant extraction methods

The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, counter current extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and distillation techniques (water distillation, steam distillation, phytonic extraction (with hydro fluorocarbon solvents). For aromatic plants, hydro water and steam distillation), hydrolytic maceration followed by distillation, expression and effleurage (cold fat extraction) may be employed. The variations in different extraction methods that will affect quantity and secondary metabolite composition of an extract depend upon: type of extraction, length of the extraction period, temperature, nature of solvent, solvent concentration and polarity. Effect of extracted plant phytochemical depends on the nature of the plant material, its origin, degree of processing, moisture content and particle size (Pandey and Tripathi, 2014).

3. MATERIALS AND METHODS

3.1. Study Area

The experimental study was conducted in two sites. *In vitro* loucicidal efficacy test was conducted in laboratory of Pathology and Parasitology Department (College of Veterinary Medicine and Agriculture, AAU) located in Bishoftu. *In vitro* accaricidal efficacy test was conducted in Ambo University, West Shewa.

3.2. Study Design

The experimental study was conducted from January 2014 to April 2015 to evaluate loucicidal and accaricidal efficacy of crude methanolic extract of *Vernonia amygdalin -a*, *Croton macrostachyus* and *Ricinus communis* leaf and petroleum ether extract of *Millettia ferruginea* seed oil against *Bovicola ovis* and *Rh. decoloratus* by using adult immersion test *in vitro*.

3.3. Study Plant Collection and Identification

Three study plants: *Vernonia amygdalina* (grawa), *Croton macrostachyus* (bisana), *Ricinus communis* (gulo) were collected from Fitche area. Fitche, Girar Jarso District, North Shewa Zone, Oromia Regional State is located in latitude and longitude of 9.50 N 36.3°E/ 9.08°N 36.6°E respectively. 114 Km North west of Addis Ababa, capital city of the country. The annual average rainfall is 1800 mm with an elevation of 2,088 meters above sea level. The annual average temperature is 21°C (NMSA, 2011). The fourth plant, seed of *Millettia ferruginea* (birbira) was collected from botanical garden of Aklilu Lemma Institute of Pathobiology in Addis Ababa University. Addis Ababa). All medicinal plant materials were selected on the basis of reports on their traditional uses against various ectoparasites (Bekele *et al*., 2012; Melaku, 2013; Karunamoorthi and Hailu, 2014). They were collected between December 2014 and January 2015. During collection latex gloves were worn to reduce possible contamination, especially by fungi. Those plants identified and verified at Aklilu Lemma Institute of Pathobiology with voucher No AG/81/05, AG/82/05, AG/83/05 and AG/84/05 resp

ectively. Leaf of *V. amygdalina*, *C. macrostachyus*, *R. communis* and seeds of *M. ferruginea* were used for loucicidal and acaricidal efficacy test against adult *B. ovis* of sheep and adult *Rh. decoloratus* of cattle *in vitro* (Annex 1 and 2).

3.4. Plant Preparation and Extraction

Collected leaves were washed with distilled water to remove dirt and soil particles. The plant were cut into small pieces, spread out on paper sheets, dried in shaded area at room temperature for two weaks and finely powdered after desiccation (Annex 3). Plant extraction, methanolic extracts of *V. amygdalina*, *C. macrostachyus* and *R. communis* leaf was conducted at Aklilu Lemma Institute of Pathobiology in Addis Ababa University, Addis Ababa. Whereas oil extraction was conducted at CVMA, AAU. 1000g dry powder of *V. amygdalina*, *R. communis* and *C. macrostachyus* leaves were soaked in methanol 1:4 in separate flask and shaken for 24h by automatic orbital shaker (Magano *et al*., 2011). The mixture was later strained using a muslin cloth and filtered using a Whatman filter paper (No. 1: 125mm) and the filtrate was concentrated in a vacuum rotary evaporator and was evaporated to dryness in an air oven at 40°C. The filtrates were stored in capped labeled bottles and kept in the refrigerator at 4°C until use (Bagavan *et al*., 2009) (Annex4).

Collected mature seeds of *Millettia ferruginea* with the pod (pale yellow in colour) were also well dried until all seeds released from the pods for 2 weak. The released seeds from the pods were collected from the sac, dried for one week to avoid moisture and coarsely powdered. A total of 1000 g of the powder material 50g at a time was uniformly packed into a thimble of a soxhlet extractor. It was exhaustively extracted with (Soxhlet) with petroleum ether (b.p. 60–80 ^{o}C) for 6h (Kumar *et al*., 2013) 12 cycles or till the solvent in the siphon tube of an extractor becomes colorless (Kumar *et al*., 2011) (Annex5). The solvent after extraction was removed by distillation on a water bath until viscous yellow colored oil was obtained (Kumar *et al*., 2013) (20g, 10 % yield). The extraction rate (%) was calculated as follows: (Eloff, 1999).

$$\text{Extraction rate (\%)} = \frac{\text{Weight of extracts (g)}}{\text{Weight of the plant material (g) before extraction}} \text{ x } 100$$

List of the plant species used and extraction and dilution solvents are depicted in table 2 below. After the plants materials were extracted, 0.5 % tween80 was used as solvent to prepare working concentrations (Annex 6).

Table 2: Plant extraction and extract dilution

Scientific Name	Local Name (Am)	Parts Used	place of Collection	Extracting Solvent	Solvent for Dilution
V. amygdalina	Grawa	Leaf	Fiche	Methanol	0.5% Teewn80
C. macrostachyus	Bisana	Leaf	Fiche	Methanol	0.5% Teewn80
R. communis	Gulo	Leaf	Fiche	Methanol	0.5% Teewn80
M. ferruginea	Birbira	Seed	Addis Ababa	Petroleum ether	0.5% Teewn80

Am = Amharic

source: **(**Bekele *et al*., 2012; Melaku, 2013; Karunamoorthi and Hailu, 2014).

3.5. Extract Dilution to Prepare Working Concentrations

The same concentration (200, 100, 50, 25, 12.5 and 6.25mg/ml/µl/ml) are used for both loucicidal and accaricidal efficacy test. 0.5 tween80 and distilled water are used as negative control while 0.1% diazinon used as positive control. Positive control, 0.1% diazinion 60 EC was diluted in water according to the manufacturers recommen dation (1:1000) before being used for further experiment (Heukelbach, *et al*, 2006). Tween 80 at concentration of 0.5% was used because at this concentration it was able to dissolve the extracts but with no harm on the parasites.

3.6. Study Parasites Collection, transportation and Identification

Lice were collected from naturally infested sheep bought from Sagure market in East Arsi Zone of Oromia National Regional State of Ethiopia. Freshly collected

parasites were transported, in plastic cups covered by cotton net gauze to allow the free circulation of air, to Pathology and Parasitology Department laboratory, CVMA at Bishoftu. Species identification was done based on morphological identification keys given by (James, 2013;Wall and Shearer, 2001). Ticks were collected from naturally infested cattle in Ambo (West Shewa Zone of Oromia, Ethiopia). Collected ticks were put in vials and the vials with ticks were wrapped in cotton net gauze for oxygen supply and transported and identify in Ambo university, Parasitol ogy laboratory. Ticks from naturally infested cattle, were identified according to (Walker *et al*., 2007). Adult *B.ovis* and *Rh. decoloratus* were used for the *in vitro* bioassays.

3.7. Adult Immersion Test

3.7.1. In vitro loucicidal efficacy test

In vitro immersion tests were carried out to evaluate the efficacy of the selected plant against *B.ovis*. The in *vitro* tests were started within 60min after lice collection (Heukelbach, *et al*, 2006). Ten active lice were counted and placed in petridish (Levot, 2000). One milliliter of each diluted extract was added to each petridish containing lice. 1ml 0.5% tween 80 and distilled water were used as negative control and untreated control. 1ml of (0.1% diazinion 60 EC) (Heukelbach *et al*., 2006). The experiment was performed in three replication for each concentration per treatment and control petridish (Gemeda *et al*., 2014). After one minute of contact time, the extracts were filtered using whatman filter paper (Khater *et al*, 2013). All plates exposed to each concentration of the plant extract and control plates were incubated at 27 ^{o}C and 80% humidity. The total incubation period was 24h (Levot, 2000). Lice were examined, under a stereoscope, after 30 min, 1hr, 2hr, 3hr, 6hr, 12hr and 24hr and death of lice were recorded in each time interval (Annex8). Death of lice was defined as the lack of limb movement, and failure to respond when the legs were stroked with a needle (Khater *et al*, 2013) (Annex7A). The percentage mortality was calculated by using a formula given elsewhere (Krishnaveni and Venkatalakshmi, 2014)

$$\text{Mortality\%} = \frac{\text{No of dead lice}}{\text{Total number of lice}}\ 100$$

Insecticidal effect of selected plant was classified as follows: strong when mortality was >80%; moderate, mortality 80–60%; weak, mortality 60–40%; little or no activity, mortality < 40%.

3.7.2. In vitro acaricidal efficacy test

Three replicates for each concentration of 10 adult ticks were dipped in the respective dilutions of extracts and control solutions for 2 min of exposure. After immersion, the ticks were recovered from tube and filtered with filter paper and placed in separate Petri dishes (Zaman *et al*., 2012). Five millilitres of 0.5% tween 80 and distilled water and (0.1% diazinion 60 EC) were used as negative control and positive control respectively. The petridish were incubated in an incubator at 30^{o}C with 80% relative humidity and death of ticks were recorded after 30 min, 1hr, 2hr, 3hr, 6hr, 12hr and 24hr interval (Annex8). The viability of ticks was checked regularly by stimulation with a needle and ticks were recorded as dead if no reaction was shown (Annex7B). The percentage mortality was calculated by the formula previously used by Krishnaveni and Venkatalakshmi (2014) as follows.

$$\text{Mortality\%} = \frac{\text{No of dead ticks}}{\text{Total number of ticks}}\ 100$$

3.8. Data Analysis

Collected raw data were stored in Microsoft Excel database system used for data management. SPSS windows version 20 were used for data analysis. Results of the study were expressed as a mean of mortality percentage ± standard error (Mean ± SE). Statistical significance was determined by one way analysis of variance (ANOVA) with multiple comparison tests (Post Hoc/Tukey's test/HSD) to compare parameter within and between groups. All significant levels set at $P<0.05$.

4. RESULTS

4.1. Physical Characteristic Features and Percentage Yield of Extracts

Physical characteristics features and percentage yield of the extracts are shown in table 3. Crude methanol extract of *V. amygdalina, C. macrostachyus* and *R. communis* leaves have dark green color, semi solid and stick nature. *M. ferruginea* seed extracted by petroleum ether have yellow colour, oil in consistency. Percentage yield was higher for *M. ferruginea* seed extract and lower for *V. amygdalina*.

Table 3: Physical characteristic features and percentage yield of selected plant extract
s

Plant species	Weight of dry powder (g)	Yield (%)	Extract Colour	Extract Consistency
V. amygdalina	1000	6.99%	Dark green	sticky and semi solid
C. macrostachyus	1000	9.68%	Dark green	sticky and semi solid
R. communis	1000	9.3%	Dark green	sticky and semi solid
M. ferruginea	1000	10%	Yellow	Liquid (oil)

4.2. *In Vitro* Loucicidal Activity of Extracts

A total of four different medicinal plants, *Vernonia amygdalina*, *Croton macrostachus* and *Ricinus communis* leaves crude extract and seed oil of *Millettia ferruginea* were tested for their loucicidal efficacy against *B. ovis.* Throughout the experimental study, negative control, (0.5% tween 80 used to dilute the extracts) and distilled water had no effect on the survival of *B. ovis*. The reference acaricide (0.1% diazinon) used as a positive control had strong efficacy against lice especially at a latter stages of exposure.

4.2.1. Loucicidal activity of Vernonia amygdalina against Bovicola ovis

Mortalities of *B. ovis* treated with different concentration of *Vernonia amygdalina* leaf extract are shown in Figure 8. Significant increase in lice mortality was started 3h post exposure with diazinon and 200 and 100mg/ml concentrations of *V. amygdalina* extract. From 6hr post exposure, diazinon has caused significantly higher lice mortality than the 200mg/ml or less concentration of the extract ($P<0.05$). Moreover, higher mortality was recorded at 24hr post exposure with 200mg/ml compared to extracts with 100mg/ml or less concentration ($P<0.05$). Generally, 0.1% diazinon had strong and 200mg/ml *V. amygdalina* extract had moderate loucicidal activity against *B. ovis*.

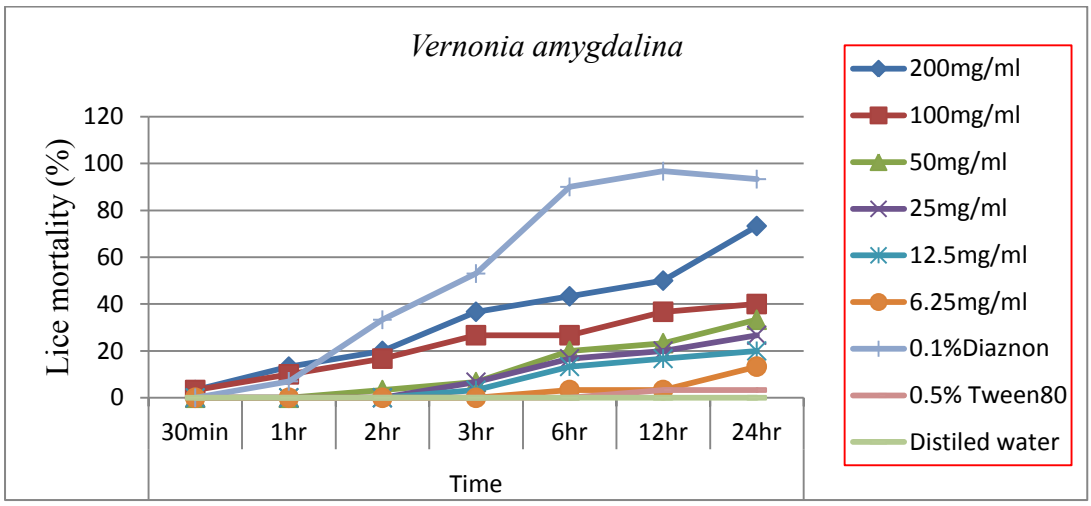

Figure 8: Mortalities of *B.ovis* treated with methanolic extract of *Vernonia amygdalina in vitro*.

4.2.2. Loucicidal activity of Croton macrostachyus against Bovicola ovis

In vitro loucicidal activity test revealed that significant increase in lice mortality was started at 2hr post exposure with diazinon and 1hr post exposure with 200, 100 and 50mg/ml concentrations of *C.macrostachyus* extract whereas for lower concentrations of the extract, it started after three hours of exposure. At 3hr post exposure, the 200mg/ml concentration of the extract had caused significantly higher lice mortality than diazinon ($P<0.05$). However, from six hours on wards, the mortality rate with diazinon and the three higher concentrations of the extract was statistically similar. After 12 and 24hr, mortality rate with 25mg/ml reaches close to those with other

higher concentrations and diazinon. Generally, 0.1% diazinon as well as extract concentrations greater than or equal to 25mg/ml of *C. macrostachyus* extract had strong loucicidal activity at 24hr post exposure. On the other hand, lower concentrations (12.5 and 6.25mg/ml) of the extract have moderate loucicidal activity against *B. ovis* after 24hr of exposure (Figure 9).

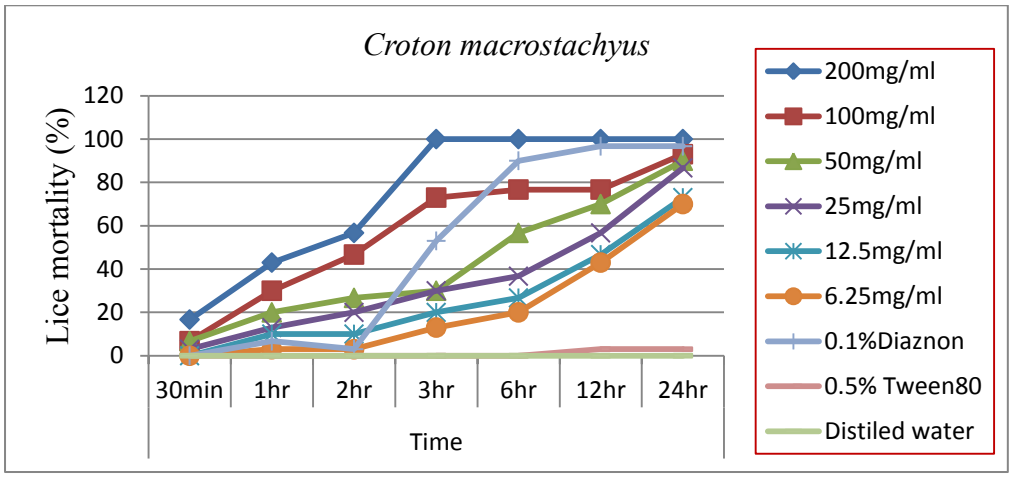

Figure 9: Mortalities of *B.ovis* treated with methanolic extract of *Croton macrostachyus in vitro*.

4.2.3. Loucicidal activity of Ricinus communis against Bovicola ovis

In vitro loucicidal activity test with *Ricinus communis* crude extract showed that significant increase in lice mortality was started 2hr post exposure with diazinon and 1h post exposure with 200 and 100mg/ml concentrations of *R. communis* extract whereas for lower concentrations of the extract, it started after six hours of exposure. Throughout the exposure time, there was no significant difference in lice mortality between diazinon and the two higher concentrations (200 and 100mg/ml) of the extract. On the other hand at of 2hr post exposure, diazinon and the two higher concentrations have resulted in significantly higher mortalities than all the lower concentrations ($P<0.05$). Generally, 0.1% diazinon as well as extract concentrations of 200 and 100mg/ml of *R. communis* extract had strong loucicidal activity at 24hr post exposure. On the other hand, all lower concentrations of the extract have fallen in the low activity classification after 24hr of exposure (figure 10).

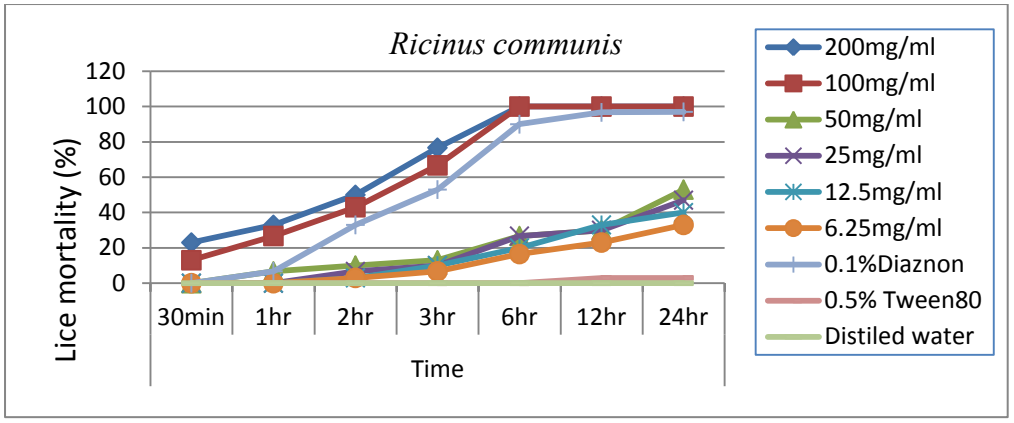

Figure 10: Mortalities of *B.ovis* treated with methanolic extract of *Ricinus communis in vitro*.

4.2.4. Loucicidal activity of Millettia ferruginea against Bovicola ovis

Millettia ferruginea revealed significant loucicidal activity as early as 1hr post exposure with all concentrations while this effect of delayed by one hour for diazinon. Between one and three hours post exposure, plant extract with 200 and 100µl/ml had caused significantly higher mortality than diazinon ($P<0.05$). Lice mortality with 200µl/ml concentration was statistically higher than for all lower concentrations whereas at three hours post exposure the highest concentration tested was only significantly more effective than concentrations less than or equal to 25µl/ml. On the other hand, as of 6h post exposure, there was no significant difference between the reference drug and all concentrations of the extract. Generally, 0.1% diazinon as well as all *M. ferruginea* extract concentrations tested had strong (>80%) loucicidal activity at 24hr post exposure (Figure 11).

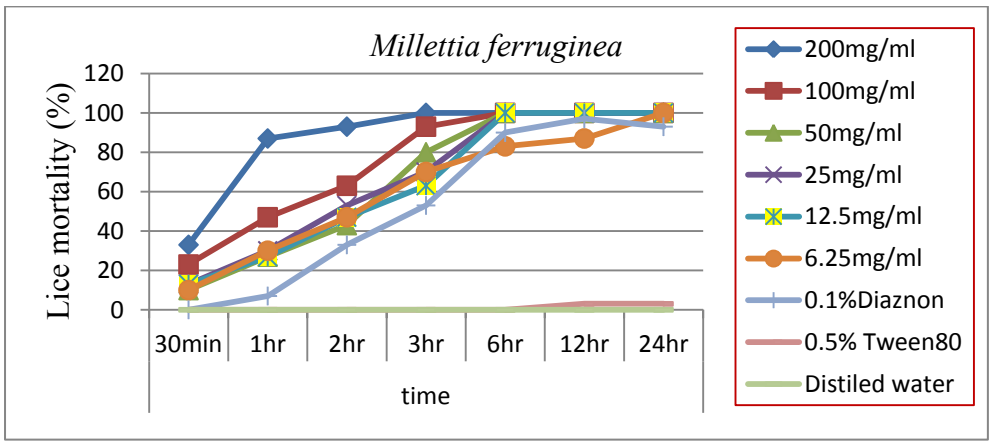

Figure 11: Mortalities of *B.ovis* treated with petroleum ether extracts of *Millettia ferruginea* seed oil in *vitro*.

4.3. *In Vitro* Acaricidal Activity of Selected Plant Extracts

A total of four different medicinal plants, *V.amygdalina*, *C. macrostachyus. R. commu nis* crude methanolic extracts and *M. ferruginea* oil extracts were tested for acaricidal activity against *Rh. decoloratus*. Throughout the experimental study, both negative control (0.5% tween 80) and the distilled water treated control had no effect on the survival of *Rh. decoloratus*. The reference acaricide (0.1% diazinon) used as a positive control had weak efficacy *Rh. decoloratus* at all time intervals tested.

4.3.1. Acaricidal activity of Vernonia amygdalina against Rh. decoloratus

Mortalities of *Rh. decoloratus* treated with different concentration of *Vernonia amygdalina* methanolic leaf extract are shown in figure 12. Significant increase in tick mortality was started 12hr post exposure with diazinon and 24hr post exposure with 200 and 100mg/ml concentrations of *V. amygdalina* extract. At 24hr post exposure period, diazinon has caused significantly higher tick mortality than the 100mg/ml or less concentration of the extract ($P<0.05$). But, no statistical difference between diazinon and 200mg/ml concentration of extracts ($P>0.05$). Moreover, there is no significant difference between the three higher concentrations tested (≥50mg/ml). Generally, both the reference acaricides (0.1% diazinon) and the methanolic extract of *V. amygdalina* had week acaricidal activity on *Rh. decoloratus*.

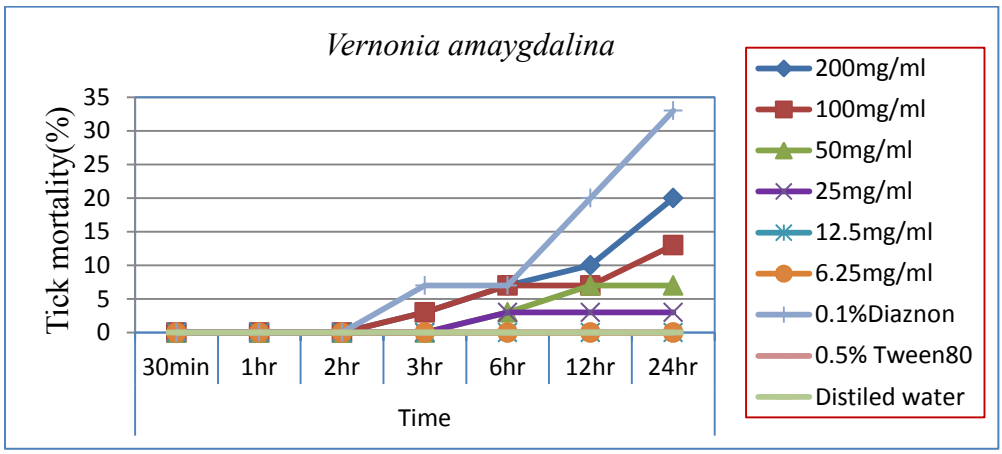

Figure 12: Mortalities of *Rh. decoloratus* treated with methanolic extract of *Vernonia amygdalina in vitro*.

4.3.2. Acaricidal activity of Croton macrostachyus against Rh. decoloratus

Figure .13 portrays mortalities of *Rh. decoloratus* treated with different concentration of *Croton macrostachyus* leaf extract. Significant increase in tick mortality was started 12hr post exposure with diazinon and 200 and 100mg/ml concentrations of *C. macrostachyus* extract. At 24hr post exposure period, diazinon and 200mg/ml concentration of the extract have caused significantly higher tick mortality than the rest of the concentrations below 100mg/ml ($P<0.05$). However, the two higher concentrations of the extract and diazinon had no significant difference in their effect on the parasite. Generally, both the reference acaricide (0.1% diazinon) and the methanolic extract of *C. macrostachyus* had week acaricidal activity on *Rh. decoloratus*.

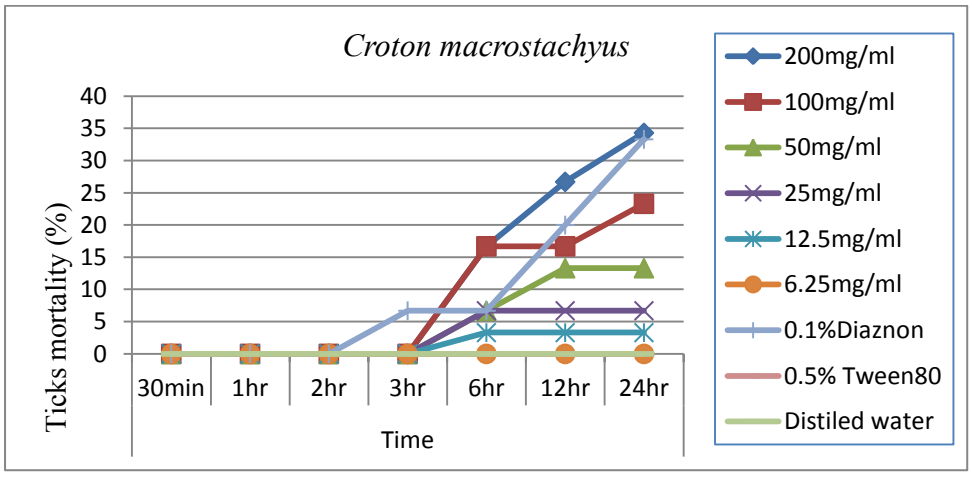

Figure 13: Mortalities of *Rh. decoloratus* treated with methanolic extract of *Croton macrostachyus* leaf *in vitro*.

4.3.3. Acaricidal activity of Ricinus communis against Rh. decoloratus

Methanolic extract of *Ricinus communis* was tested against *Rh. decoloratus in vitro.* Significant increase in tick mortality was started 2hr post exposure with 200mg/ml concentration of extract, 12hr post exposure with 100mg/ml concentration of the extract and 12hr post exposure with diazinon. Starting from 1hr post exposure the 200mg/ml concentration of *R. communis* extract has caused significantly higher mortality compared to the diazinon ($P<0.05$). It had also statistically higher activity than the 100mg/ml concentration as of 3h post exposure. On the other hand, mortality

rate with diazinon has no significant difference compared to all plant extract concentrations below 200mg/ml. Generally, this extract has appreciably significant advantage over the reference acaricide at all time intervals (Figure 14).

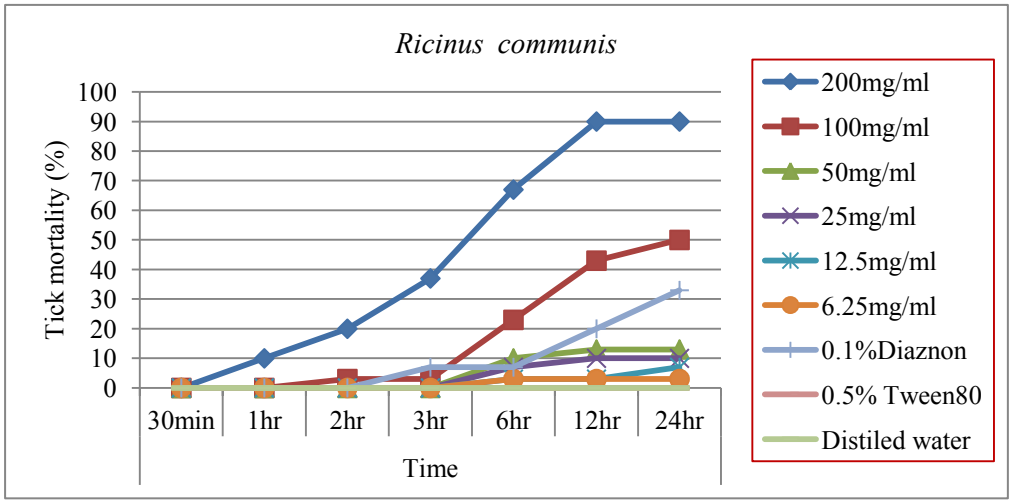

Figure 14: Mortalities of *Rh. decoloratus* treated with methanolic extract of *Ricinus communis in vitro.*

4.3.4. Acaricidal activity of Millettia ferruginea against Rh. decoloratus

Petroleum ether extract of *Millettia ferruginea* seed oil was tested against *Rh. decoloratus in vitro.* Significant increase in tick mortality was started 3hr post exposure with 200 and 100µl/ml concentration of extract, 6hr post exposure with 50µl/ml extract and 12hr post exposure with 25µl/ml concentration of the extract and with diazinon. There was no difference between the two higher concentrations tested in causing mortality as of 3h pot exposure of the tick. On the other hand, at 24hr post treatment, efficacy of diazinon was statistically much lower than that of plant extracts with 50, 100 and 200µl/ml concentrations ($P<0.05$). Generally, this extract has appreciably significant advantage over the reference acaricide at all time intervals starting from 6hr of exposure (Figure 15).

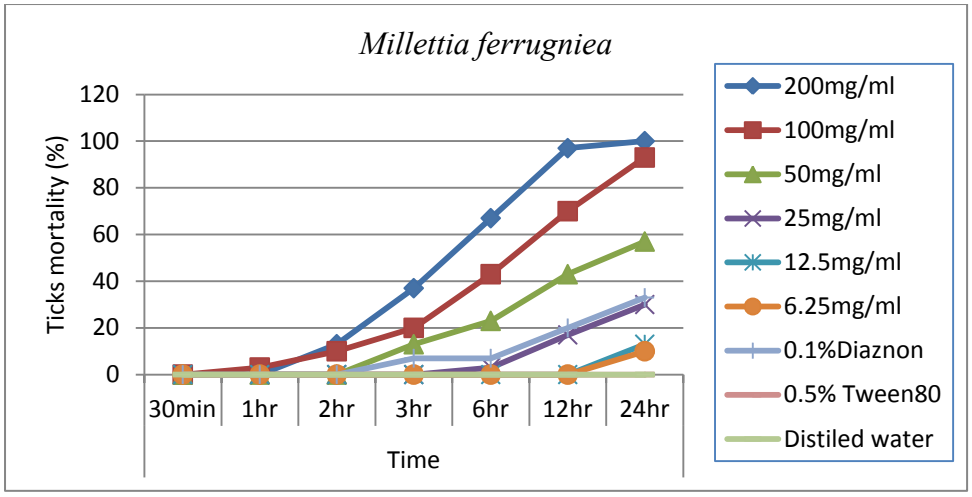

Figure 15: Mortalities of *Rh. decoloratus* treated with petroleum ether extract of *Millettia ferruginea* seed oil *in vitro*.

4.4. Comparative Efficacy of Extracts

4.4.1. Between plant comparisons

Before 3hr of exposure only 200µl/ml concentration *M. ferruginea* showed (strong loucicidal activity at 1hr (86.7%) and 2h (93.3%) of exposure and moderate loucicidal activity at 2hr (63.3%) of exposure to 100µl/ml), all concentration of the other plant extracts had low loucicidal activity. Except for *V. amygdalina*, at two higher concentrations of all the remaining plants at all time intervals from 3hr to 24hr of exposure had strong to moderate impact on *B.ovis. V. amygdalina* has shown moderate activity at 200mg/ml concentration only at 24h post exposure. On the other hand, when lower concentrations of the extracts was compared, *M. ferruginea* stands first since it caused moderate to strong loucicidal activity as of 3hr post exposure whereas *C. macrostachyus* had moderate to strong activity only starting from 12hr post exposure (Table 4A and B). Other plants had minimal effect on the parasite at lower concentrations.

The effect the extracts on tick showed a different scenario. Before 6hr of exposure time all plant extracts exhibited lower acaricidal activity at all concentrations. After 6hr exposure time 200µl/ml of *M. ferruginea* and *R. communis* (mg/ml) were more effective against *Rh. decoloratus* than *C. macrostachyus* and *V. amygdalina.* 100 and

50µl/ml concentration of *M. ferruginea* oil have significantly more activity against *Rh. decoloratus* ticks than *R. communis* with the same concentrations and the three lower concentration ≤25mg/ml/µl/ml of *M. ferruginea* and *R. communis* have low accaricidal activity at 12 and 24 hr of exposure time.

Table 4A: Comparisons of loucicidal activity of selected medicinal plants against *Bovicola ovis* at 3h and 6h exposure.

conc. (mg/ml)	Mortality (%)± SE							
	3hr				6hr			
	V.A	C. *M*	*R. C*	*M.F*	*V.A*	C. *M*	*R. C*	*M.F*
	(µl/ml)							
200	36.7±6.7^{a}	100±0.0^{b}	76.7.3±8.8^{bc}	100±0.0^{cbd}	43.3±3.3^{a}	100±0.00^{b}	100±0.0^{bc}	100±0.0^{bcd}
100	26.7±3.3^{a}	73.3±13.3^{b}	66.7±3.3^{bc}	93.3±3.3^{bcd}	36.7±3.3^{a}	76.7±12^{b}	100±0.0^{bc}	100±0.0^{bcd}
50	6.7±3.3^{a}	30±10^{b}	13.3±6.7^{ac}	80±5.7^{d}	20±5.8^{a}	56.7±26^{ab}	16.7±3.3^{abc}	100±0.0^{d}
25	6.7±3.3^{a}	30±5.8^{ab}	10±0.0^{abc}	70±5.7^{d}	16.7±6.7^{a}	36.7±6.7^{ab}	26.7±8.8^{abc}	100±0.0^{d}
12.5	3.3±3.3^{a}	20±0.0^{b}	10±5.8^{ac}	63.3±3.3^{d}	13.3±3.3^{a}	26.7±6.7^{ab}	26.7±3.3^{abc}	100±0.0^{d}
6.25	0±0.00^{a}	13.3±3.3^{ab}	6.7±6.7^{abc}	70±11.5^{d}	3.33±3.3^{a}	20±5.8^{ab}	16.7±8.8^{abc}	83.3±12^{d}

V.A =Vernonia amygdalina, C. M = Croton macrostachyus, R. C= Ricinus communis leaf and *M.F= Millettia ferruginea*

Values are expressed as mean of mortality parentage ± SE. Mortality parentage values with different letters in the same raw for each time exposure are significantly different ($P < 0.05$).

Table 4 B: Comparisons of loucicidal activity of selected medicinal plants against *Bovicola ovis* at 12h and 24h exposure

conc. (mg/ml)	Mortality (%) ± SE							
	12h				24h			
	V.A	*C. M*	*R. C*	*M.F*	*V.A*	*C. M*	*R. C*	*M.F*
				(µl/ml)				
200	50.0±5.8^{a}	100±0.0^{b}	100±0.00^{bcd}	100±0.0^{bc}	73±6.7^{a}	100±0.0^{b}	100±0.00^{bc}	100±0.0^{bcd}
100	36.7±3.3^{a}	76.7±12^{b}	100±0.00^{bcd}	100±0.0^{bc}	40±5.8^{a}	93±3.3^{b}	100±0.00^{bc}	100±0.0^{bcd}
50	23.3±8.8^{a}	70±17^{ab}	26.7±6.7^{abc}	100±0.0^{bd}	33.3±6.7^{a}	90±5.8^{b}	53±3.3^{ac}	100±0.0^{bd}
25	20±5.8^{a}	56.7±14.5^{ab}	30.0±5.8^{ab}	100±0.0^{d}	26.7±3.3^{a}	86.7±3.3^{b}	46.7±14.5^{ac}	100±0.0^{bd}
12.5	16.7±3.3^{a}	43.3±13.3^{ab}	33.3±8.8^{ab}	100±0.0^{d}	20±0.0^{a}	70±0.0^{b}	40±10.0^{ac}	100±0.0^{d}
6.25	3.3±3.3^{a}	46.7±12^{ab}	23.3±3.3^{ab}	86.7±8.8^{d}	13±3.3^{a}	70±0.0^{b}	33.3±6.7^{c}	100±0.0^{d}

V.A =Vernonia amygdalina, C. M = Croton macrostachyus, R. C= Ricinus communis leaf and M. F= Millettia ferruginea

Values are expressed as mean of mortality % ± SE. Mortality % values with different letters in the same raw are significantly different ($P < 0.05$).

4.4.2. Between Parasite Comparisons

At 24 hours post exposure, even higher concentration of *V. amygdalina* and *C. macrostachyus* had no strong significant effect on *Rh. decoloratus* whereas they had moderate and strong to efficacy on *B.ovis* respectively at higher concentrations of 200mg/ml. On the other hand, although the effect of *R. communis* and *M. ferruginea* was minimal on ticks at early exposure and lower concentrations, they have similar strong loucicidal and acaricidal efficacy at 24h post exposure at higher concentrations of 200mg/ml and both 200 and 100µl/ml respectively .

5. DISCUSSION

The present study was aimed to assess the loucicidal and acaricidal efficacy of extracts of four plant species and compare their efficacy against selected ectoparasites species. Diazinon was used as a positive control to evaluate the efficacy.

5.1. Loucicidal Efficacy of Plant Extracts

Our study revealed that except *V. amygdalina*, extracts of the other three species of plants demonstrated highly appreciable loucicidal activity at different concentrations. *V. amygdalina* had moderate activity after 24 hours of exposure. As far as our literature search is concerned, no study was reported on loucicidal efficacy of *V. amygdalina*. However, leaf extracts of the plant have been reported to have an effect on *Spodoptera litura* (maize pest) and *Culex pipiense pallense* (Ohigashi *et al*., 1991).

Even though, there is no study conducted on loucicidal activity of methanol extract of *C.macrostachyus*, the present finding is in line with Karunamoorthi *et al*. (2010) who showed methanolic crude leaf extract of *C. macrostachyus* exhibited larvicidal activity against late third instar larvae of *Anopheles arabiensis*, a potent malaria vector in Ethiopia. In addition, phytochemical characterization showed that *C. macrostachyus* contains flavonoids, alkaloids, tannins, saponins, terpenes, terpenoides, steroids, essential oil and volatile components (Yibralign, 2007;Okeleye *et al*., 2011). These and other constituents of the plant may be responsible for the toxic effect of the extracts that caused mortality of *B.ovis*.

Strong loucicidal activity of crude methanolic extracted *R. communis* at 200mg/ml and 100mg/ml concentration was demonstrated in the present study and its effect was equivalent to the commercial acaricide, diazinon. It has been reported that the high toxicity of *R. communis* seed extracts to *P. xylostella* (pest of cruciferous crops) was due to the presence of ricin in the extracts (Tounou *et al*., 2011) and ricin is reported as one of

the most poisonous natural compounds (Kozlov *et al*., 2006; El-Nikhely *et al*., 2007). However, ricin concentration has been reported to be less in leaves than in seed of *R. communis.* Moreover, previous phytochemical studies reported that the crude extract of leaves of the plant was positive for saponins, flavonoids, glycosides, fixed oils and fats, while negative for alkaloids, carbohydrate, tannins and phenolic compound and proteins and amino acids (Kumar *et al*., 2011). Mandal (2010) also reported that *R. communis* seed extracts provided an excellent potential against the *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes albopictus* mosquito vector.

Similarly, our findings clearly revealed that all concentrations of *Millettia ferruginea* seed oil had pronounced loucicidal efficacy comparable to the positive control (diazinon). this report is in agreement with Getahun and Jembere (2006) who reported 93–100% mortality of adult *Macrotermes termites* by water extracts of *M. ferruginea* seed powder at 10–40%w/v concentration suggesting that it has potent insecticidal activity. Compared to our other plant extracts, the activity of this extract at a very low concentration deserves special attention as it can be the first candidate to investigate lead compounds. The presence of flavonoids, isoflavonoids, chalcones (Bekele, 1988) and rotenone (Dagne and Bekele, 1990) was previously confirmed in *M. ferruginea* seed. Rotenone, a non-specific botanical insecticide with some acaricidal properties, is not only used in home gardens for insect control, but also used for lice and tick control on pets and for fish eradications as part of water body management (Jembere, 2002; Hien *et al*., 2003).

5.2. Acaricidal Efficacy of Extracts

The results obtained in the present study indicated that *V. amygdalina* and *C. macrostachyus* had no significant acaricidal efficacy on *Rh. decoloratus*. However, methanolic extract of *R. communis* leaf had strong accaricidal activity (90%) against the tick at 200mg/ml concentration. In line with this finding, Kumar *et al*. (2011) and Ghosh *et al*. (2013) have reported that similar methanolic extract of the plant has potent activity on *Rh. decoloratus* even at lower concentrations. Absence of efficacy at lower concentration in our study may be attributed to differences in methodology or the species

of parasite against which the extracts were tested. It could also be related to the nature of the plant at the time of collection (Pandey and Tripathi, 2013). Moreover, previous phytochemical studies reported that the crude methanol extract of *R. communis* leaves was positive for saponins, flavonoids, glycosides, fixed oils and fats, while negative for alkaloids, carbohydrate, tannins and phenolic compound and proteins and amino acids (Kumar *et al*., 2011). Surprisingly, the extract of the plant had much higher efficacy compared to the commercial compound, diazinon putting the latter under suspect for acaricidal resistance.

Similar to *R. communis*, higher concentrations of *M. ferruginea* seed oil had strong accaricidal activity which was much better than the effect of diazinon. This is in concordance with Kumar *et al.* (2013), who reported 100% mortality of *Amblyomma variegatum larvae* even at concentrations as low as 20% of the seed oil extracted with petroleum ether. Secondary metabolites such as flavonoids, isoflavonoids, chalcones and rotenone have previously been reported from the seed extract (Jembere *et al*., 2007; Dagne and Bekele, 1990; Bekele, 1988).

6. CONCLUTIONS AND RECOMMENDATIONS

Extracts of *V. amygdalina*, C. *macrostachyus, R. communis* and *M. ferruginea* were tested against *B. ovis* and *Rh. decoloratus* for their killing efficacy at different concentrations and time intervals. It was observed that except *V. amygdalina*, which had moderate loucicidal activity, all the three plants had strong loucicidal activity greatly comparable to the effect of 0.1% diazinon at higher concentrations. Moreover, *R. communis* and *M. ferruginea* had much better efficacy against the ticks, *Rh. decoloratus*, than the reference acaricide, diazinon. Extracts of the remaining two plants had no significant efficacy on the tick species at all concentration and maximum time tested. Efficacy of the extracts increases with increasing concentration and exposure time. Therefore, the present study concluded that C. *macrostachyus*, *R. communis* and *M. ferruginea* against *B. ovis* whereas *R. communis* and *M. ferruginea* against *Rh. decoloratus* could be used as potential alternative to substitute commercially available drugs.

Based on the above concluding remarks, the following recommendations are forwarded:

- Further study is recommended to elucidate the acaricidal effect of the selected plants by using different extraction methods and other parts of the plant.
- To determine the active compounds responsible for the killing effect of the potent extracts, it is essential to fractionate the extracts and test each component separately.
- For those extracts that have shown promising results, *in vivo* toxicity and efficacy test should be done to validate the importance of the materials for future development of lead compounds towards producing alternative acaricides.
- The low efficacy of diazinon on ticks needs attention. Hence, acaricidal resistance study against both the tested tick species and other species of ticks is recommended.

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8. ANNEXS

Annex 1: Plant collection format

Name of plant	Local name of plant	Parts of plant collected	place of collection

Annex 2: Pictures of plants used in the loucicidal and acaricidal Activity tests

Vernonia amygdalina

Croton macrostachyus

Ricinus communis

Millettia ferruginea seed

Annex 3: Pant material preparation

- Collected plant part were washed with distilled water to remove dirt and soil particles.
- The plant were cut into small pieces, spread out on paper sheets, dried in shaded area at room temperature.
- Completely desiccated plant part powdered finely.

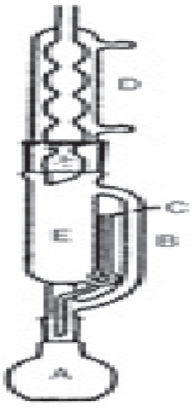
Annex 4: Crude extraction procedure

- ✓ Powdered plant material Weighed, transfered in separate flask and extracting agent added .
- ✓ Each flask contain plant material and extracting agent shaken for 24h by automatic orbital shaker.
- ✓ The mixture was later strained using a muslin cloth and filtered using a Whatman filter paper (No. 1: 125mm) .
- ✓ The filtrate was concentrated in a vacuum rotary evaporator and was evaporated to dryness in an air oven at 40°C .

Annex 5: Oil extraction method

- The finely grounded *Millettia ferruginea* seed placed in thimble made of strong filter paper which is placed in chamber E of the Soxhlet apparatus.
- The extracting solvent in flask A is heated, and its vapors condense in condenser D.
- The condensed extractant drips into the thimble containing the powdered seed, and extracts it by contact. When the level of liquid in chamber E rises to the top of siphon tube C, the liquid (oil) contents of chamber E siphon into flask A.

- This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated.

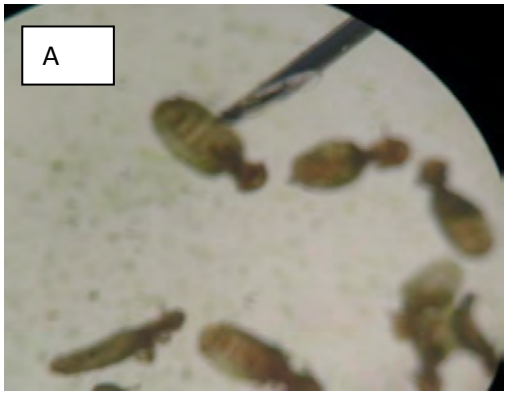


Soxhlet apparatus

Annex 6: Extract physical characteristics , yield and dilution format recording format

Name Of plant	Colour of extracted product	Characteristics of extracted material	Extraction rate (%)	Diluting agent

Annex 7: Extract efficacy testing method by cheking mortality of lice and ticks



Annex 8: *In vitro* loucicidal and acaricidal efficicacy test recording format

Plant	Concentration	Replication	No immersed lice or ticks	No dead parasite after/post incubation						
				30min	1hr	2hr	3 hr	6hr	12hr	24hr
	20%	1	10							
		2	10							
		3	10							
	10%	1	10							
		2	10							
		3	10							
	5%	1	10							
		2	10							
		3	10							
	2.5%	1	10							
		2	10							
		3	10							
	1.25	1	10							
		2	10							
		3	10							
	0.625%	1	10							
		2	10							
		3	10							
Positive control	0.1% Diazinon	1	10							
		2	10							
		3	10							
Negative control	Tween80	1	10							
		2	10							
		3	10							
	Distilled water	1	10							
		2	10							
		3	10							