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IN VITRO LOUSCIDAL AND ACARICIDAL ACTIVITIES OF ALKALOID OF *CALPURNIA AUREA* AND FRACTIONS OF *RICINUS COMMUNIS* EXTRACTS AGAINST *LINOGNATHUS OVILLUS* AND *AMBLYOMMA VARIEGATUM*

MSc THESIS

BY

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JUNE, 2016

BISHOFTU, ETHIOPIA

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Thesis submitted to College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirement for degree of Masters of Science in Tropical Veterinary Parasitology

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SIGNATURE PAGE

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Parasitology and Pathology

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DEDICATION

I dedicate this MSc thesis to my step mother Chaltu Ayana; I dreamed a little dream, Once upon a time. I dreamed we'd be together one day, Sweet mammy of mine. Sadly that dream was not meant to be, and it's very difficult to know, that now you won't be coming to me. You weren't strong enough to thrive and grow, but I know that you're in heaven now and that's a very good place to be. And I know that when I get there, I'll recognize you, and you'll know me. We'll get to share the love we would have shared here on this earth. And then we'll know without a doubt what all this waiting was worth.

STATEMENT OF THE AUTHOR

First, I affirm that this thesis is my unalloyed 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 **PAGES**

SIGNATURE PAGE **1**

APPROVAL SHEET .... **II**

DEDICATION .... **III**

STATEMENT OF THE AUTHOR .... **IV**

TABLE OF CONTENTS .... **VI**

ACKNOWLEDGEMENTS .... **IX**

LIST OF TABLES .... **X**

LIST OF FIGURES .... **XI**

LIST OF APPENDICES .... **XII**

ABBREVIATIONS .... **XIII**

ABSTRACT .... **XIII**

1. INTRODUCTION **1**

2. LITERATURE REVIEW **4**

2.1. Ectoparasites and Impact of their Infestation **4**

2.1.1. Lice and lice infestation *4*

2.1.2. Ticks and tick Infestation *8*

2.2. Ectoparasites Control Methods **11**

2.3. Challenges of Ectoparasites Control **12**

2.4. Alternative Approaches to Chemical Control **13**

2.5. Herbs in Ethno-Veterinary Medicine **14**

2. 5.1. Study plants *15*

3. MATERIALS AND METHODS **19**

3.1. Study Area **19**

3.2. Study Design **19**

TABLE OF CONTENT (*Continued*) **PAGES**

3.3. Study Materials 19

3.3.1. Study parasites 19

3.3.2 Study plant 20

3.4. Plant Extraction 21

3.4.1. Solvent-solvent fractionation of R. communis extracts 21

3.4.2. Extraction and isolation of alkaloids from C. aurea 21

3.4.3. Analytical Thin Layer Chromatography (TLC) 22

3.5. *In Vitro* Louscidal and Acaricidal Efficacy Tests 22

3.6. Data Analysis 24

4. RESULTS 25

4.1 Physicochemical Characteristics and Yield of Plant Extracts 25

4.1.1. Phytochemical screening of solvent fractions of Ricinus communis leaf extract 27

4.1.2. TLC of solvent fractions of Ricinus Communis leaf extract 28

4.1.3. TLC of the alkaloid extract of Calpurnia aurea 29

4.2. *In Vitro* Louscidal Activity of *Calpurnia aurea* and *Ricinus communis* 29

4.2.1 In vitro louscidal activity of alkaloid extract of Calpurnia aurea 29

4.2.2. Evaluation of In vitro louscidal activity of acetone extracts of Ricinus communis 31

4.2.3. In vitro louscidal activity of methanol fraction of Ricinus communis 31

4.2.4. In vitro louscidal activity of chloroform fraction of Ricinus communis 34

4.2.5. In vitro louscidal activity of the petroleum ether fraction of Ricinus communis 34

4.3. *In vitro* Acaricidal Activity of *Calpurnia aurea* and *Ricinus communis* 37

4.3.1. In vitro acaricidal activity of alkaloid extract of Calpurnia aurea on Amblyomma variegatum 37

4.3.2. In vitro acaricidal activity of the acetone fraction of Ricinus communis extracts 38

4.3.3. In vitro acaricidal activity of the methanol fraction of Ricinus communis.................39

TABLE OF CONTENT (*Continued*) **PAGES**

4.3.4. In vitro acaricidal activity of chloroform fraction of Ricinus communis 41

4.3.5. In vitro acaricidal activity of petroleum ether fractions of Ricinus communis 41

4.4.2. Acaricidal and louscidal activity comparison between Calpurnia aurea alkaloid and solvent fractions of Ricinus communis..45

5. DISCUSSION 48

6. CONCLUSION AND RECOMMENDATIONS 52

7. REFERENCES.. 53

8. APPENDICES 67

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LIST OF TABLES **PAGES**

Table 1: Physical characteristics and percent yield of the various solvent extracts of *Ricinus communis* and the alkaloid extract of *Calpurnia aurea* 26

Table 2: Phytochemical screening results of the various solvent extracts of *Ricinus communis* 27

Table 3: Louscidal activity of different concentration of alkaloid extract of *Calpurnia aurea* on *Linognathus ovillus* at different times of exposure 30

Table 4: Louscidal activity of different concentrations of the acetone and methanol fractions of *Ricinus communis* against *Linognathus ovillus* at different times of exposure 33

Table 5: Louscidal activity of the different concentrations of petroleum ether and chloroform fractions of *Ricinus communis* against *Linognathus ovillus* at different times of exposure 35

Table 6: Acaricidal activity of different concentrations of the alkaloid extract of *Calpurnia aurea* on *Amblyomma variegatum* at different times of exposure 38

Table 7: Acaricidal activity of the different concentration of acetone and methanol fractions of *Ricinus communis* against *Amblyomma variegatum* at different times of exposure...... 40

Table 8: Acaricidal activity of different concentration of chloroform and petroleum fractionated extract of *Ricinus communis* against *Amblyomma variegatum* at different times of exposure 43

Table 9: Comparison between extract effect of louscidal and acaricidal of alkaloid of *Calpurnia aurea* and fractions of *Ricinus communis* 45

Table 10: LC_{50} and LC_{90} with 95 % CI of alkaloid extract of *Calpurnia aurea* and solvent fractions of *Ricinus communis* obtained by AIT against *Amblyomma variegatum* and *Linognathus ovillus* after 24hrs time exposure 47

LIST OF FIGURES **PAGES**

Figure 1: *Calpurnia aurea* (Ait.) Benth tree 16

Figure 2: *Ricinus communis* L. tree 18

Figure 3: TLC chromatogram of the various solvent extracts of *Ricinus communis* 28

Figure 4: TLC chromatogram of the alkaloid extract of *Calpurnia aurea* 29

Figure 5: Log (Dose) - Probit Plot of *Linognathus ovillus* after 24hrs exposure to alkaloid extract of *Calpurnia aurea* and solvent fractions of *Ricinus communis* 36

Figure 6: Log (Dose) - Probit Plot of *Amblyomma variegatum* exposed various dose of alkaloid extract *Calpurnia aurea* and solvent fractions of *Ricinus communis* after 24hr time exposure........ 44

LIST OF APPENDICES **PAGES**

Appendix 1: TLC procedure followed during *Ricinus communis* extract 67
Appendix 2: Preliminary phytochemical screening of solvent extracts 67
Appendix 3: Picture taken during plant collection and drying of Study plant 69
Appendix 4: Picture of some of extraction equipment used for plant extraction 70
Appendix 5: Picture of some phytochemical screening of *Ricinus communis* extracts............. 71
Appendix 6: Picture taken during collection and bioassay of parasite 72
Appendix 7: *In vitro* louscidal and acaricidal efficacy test recording format 74

ABBREVIATIONS

AAU	Addis Ababa University
AIT	Adult Immersion Test
CA	*Calpurnia aurea*
CSA	Central Statistics Authority
CDC	Centers for Disease Control and Prevention
DDT	Dichloro-Diphenyl-Trichloro-Ethane
DEET	N,N-Diethyl-m-Toluamide
DMSO	Dimethyl Sulphuxode
EVM	Ethno Veterinary Medicine
GDP	Gross Domestic Product
MOARD	Ministry of Agricultural and Rural Development
MOFED	Ministry of Finance and Economic Development
NTHNC	National Travel Health and Network Centre
NMR	Nuclear Magnetic Resonance
OIE	Office International des Epizooties
RC	*Ricinus communis*
TLC	Thin Layer Chromatography
USD	United States Dollar

ABSTRACT

The present study was designed to evaluate the louscidal and acaricidal activities of alkaloids of *Calpurnia aurea* and fractions of *Ricinus communis* leaves extracts. Alkaloid of *C. aurea* and

solvent fractions of *R. communis* leaves extract at concentrations of 200, 100, 50, 25, 12.5 and 6.25 mg/ml were used for *in vitro* adult immersion test of ticks and lice, which then were monitored for their mortality rates for 24hrs. The activities of test substances were evaluated against *Amblyomma variegatum* and *Linognathus ovillus,* and compared with diazinon 60 EC. After 24hrs post exposure, two higher concentrations of 200 and 100 mg/ml of the alkaloid extract caused 100±0.5% and 100±0.6% lice mortality, and 100±0.33 and 93.3±0.33 tick mortality respectively. The alkaloid extract showed an insignificant difference in its acaricidal and louscidal activity when compared to the Diazinon 60EC at the same concentration ($P > 0.05$). LC_{50} and LC_{90} values (with 95% confidence limits) of the alkaloid of *C. aurea* for lice and tick were estimated 9.08 mg/ml (6.21-13.47), 17.65 mg/ml (11.71-22.49) and mg/ml 16.69 (11.77, 26.64), 31.69 mg/ml (21.25-50.72), respectively. Based on LC_{50} and LC_{90} values, alkaloid extract was found to be more effective in killing of lice than ticks. Dose response data of *C. aurea* alkaloid extract on *L. ovillus* and *A. variegatum* indicated the gradual increase in the mortality pattern with slopes of 3.1188, and 3.2321, and R^2 values of 0.9702 and 0.9882 suggesting that 97.02, 98.82% data were correlated with log concentration, respectively. Chloroform and petroleum ether fractions of *R. communis* extracts showed less louscidal and acaricidal effects than the acetone and methanol fractions extracts based on the calculated LC_{50} and LC_{90}. At higher concentration of 200 mg/ml, the chloroform and petroleum ether fractions extracts showed weaker activity on both *A. variegatum* and *L. ovillus* with significant difference ($P < 0.05$) when compared to that of diazinon after 24hr post exposure. As compared to the alkaloid extract of *C. aurea*, fractions of *R. communis* extracts were less lethal both to lice and tick. Alkaloid of *C. aurea* and fractions of *R. communis* extract were shown to induce tick and lice mortality in a time and dose-dependent manner. The results obtained in this study indicate that the alkaloid extract of *C. aurea* and the polar solvent fractions of *R. communis* have promising louscidal and acaricidal activities, lending support for further investigation of the plants to isolate the active components.

Keywords: *Acaricide, Alkaloid, Ambylomma, Calpurnia aurea, Louscidal, Ricinus communis*.

1. INTRODUCTION

In Ethiopia, agricultural development is considered a priority by the Government for stimulating overall economic growth, reducing poverty and achieving food security. The agricultural sector of Ethiopia accounts for about 42% of GDP, more than 80% of export, and 85% of employment (MOFED, 2011). Within agriculture, the livestock subsector provides an opportunity for further development. The share size of the national livestock herd, being one of the largest in Africa, makes it resource with the potential to contribute significantly to national development, including to poverty reduction. Moreover, about 53 million cattle, 27.35 million sheep and 28.16 million goats are estimated to be found in the country, which is one of the largest and most diverse in Africa (CSA, 2014).

Hides from cattle and skins from goats and sheep are important economic products contributing for the largest share to the total and agricultural export commodities (FAO, 2005) followed by live animals (Ayele *et al*., 2003). Based on annual off take rates of 7% for cattle, 33% for sheep and 35% for goats, the potential production is estimated at 3.78 million cattle hides, 8.41 million sheep skins and 8.42 million goat skins in 2012/2013 (CSA, 2013). However, their contribution to food production, rural income and export income are still far below the expected potential. This is because small ruminants particularly sheep production in Ethiopia is constrained by the compound effects of diseases, poor feeding and poor management (Hirpa and Abebe, 2008).

Skin diseases are known to affect the quality of skin. In 1996/97 six tanneries that are found in and around Addis Ababa has rejected 2,037,745 pieces of skins, which caused loss of 6.3 million USD (Kassa, 1998) and in 1998/99 three tanneries that are found in Amhara region have reported 443,602 pieces of skin rejection per annum, which worth 1.4 million USD (MoARD, 2005).

The infestation of lice on sheep and goats has been reported from different parts of Ethiopia. Lice were considered as causes of cockle following keds and are visible on the skin surface of affected animals leading to skin rejection (Kidanu, 2001). Apart from the occurrence of lice in small ruminants, higher percentages of tick infestation on cattle have been documented. In Ethiopia, ticks occupy the first place amongst the external parasites by the economic loss they incurred when they infest livestock particularly cattle (Bekele, 2002).

Ectoparasites treatment with various acaricides like diazinon, fenvalerate, deltamethrin and avermectin has been attempted with different grades of success. Rapid development of resistance (Clark *et al*., 1996), high cost and environmental contamination (O'Brien, 1999) and health hazards to humans during treatment of animal (Das *et al.*, 2010) are, however, the major problems associated with the use of synthetic acaricides. Concern about toxicity (O'Brien, 1999) of many acaricides limits their use and reduces the number of safe effective products available. These problems have led to research efforts to discover new effective compounds.

The identification of novel active plant derived natural compounds could increase the number of available chemotherapeutic agents, thereby reducing the frequency of development of resistance and providing alternative drugs with greater acceptance, especially in terms of environmental safety (Alawa *et al*., 2003). These shortcomings have prompted the search for alternative ectoparasites control methods that are cheap and environmentally friendly like plant extracts (Zorloni *et al*., 2010).

Different crude extract of indigenous plants like *Calpurnia aurea* and *Ricinus communis* have shown killing capacity against *Bovicola ovis* and *Amblyomma variegatum in vitro* (Askale, 2015; Sisay, 2015). However, information on activities of this plant extract on *Linognathus ovillus* and, which active ingredient is responsible for the killing activity is lacking.

In view of all the above back ground, use of botanical acaricides against highly pathogenic and economically important ectoparasites like ticks, mites and lice is extremely important.

Therefore, the objectives of the present study were:

- To evaluate *in vitro* louscidal and acaricidal activities of the alkaloid of *Calpurnia aurea* and fractions of *Ricinus communis* leaves extract against *Linognathus ovillus* and *Ambylomma variegatum;*
- Compare acaricidal and louscidal activities of the isolated alkaloid extract and fractions obtained against *Linognathus ovillus and Amblyomma variegatum*.

2. LITERATURE REVIEW

2.1. Ectoparasites and Impact of their Infestation

The term ectoparasites or external parasites refer to "parasites, with few exceptions, that live or burrow into the surface of their host's epidermis" (Wall and Shearer, 2001). Ectoparasites acquire blood meal from their host without penetrating the entire body of their host. The association between arthropod ectoparasites and vertebrate hosts may take on a 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).

The effect of skin parasitism usually depends on the size of invading population, on the manner on which the parasite ekes out its existence and the state of nutrition of the host animal when infected. The damage ectoparasites inflict may be mechanical, but the situation is complicated also by host reactions to the presence of the particular parasite, their secretion and excretion (Peter, 1995).

2.1.1. Lice and lice infestation

The lice belong to the order *Phthiraptera* which is divided into four suborders; *Anoplura, Amblycera, Ischnocera and Rhynchophthirina Amblycera* and *Ischnocera* are known as chewing lice while *Anoplura* are described as sucking lice (Wall and Shearer, 2008). *Rhynchophthirina* is a very small suborder that includes just two species, one of which is a parasite of elephants and the other for warthogs (Wall and Shearer, 2001).

Out of more than 50 species of *Linognathus* described, six occur on domestic animals. The species that parasitize sheep and goats includes; the face louse *L. ovillus*, the foot louse, *L. pedalis*; the goat sucking louse, *L. stenopsis*; and the closely related species, *L. africanus* on sheep and *L. stenopsis* and *L. africanus* on goats (Wall and Shearer, 2001). Members of this family do not have eyes or ocular points. The second and third pairs of legs are larger than the first pair and end in stout claws. In species of *Linognathus* the thoracic sternal plate is absent or weakly developed. Parasternal plates are absent from the abdomen. Adult female lays a single egg per day. Eggs hatch in 10- 15 days; giving rise to nymph which requires about 2 weeks passing through three nymphal stages. The egg to adult life cycle requires about 20 – 40 days (Wall and Shearer, 2001).

The face louse, *L. ovillus*, usually occurs in colonies on the ear and face of sheep. The preferred sites for *L. pedalis* are the feet, legs and scrotum. At high densities however, both species may spread over the entire body. *L. pedalis* can survive for several days off the host. So the infestation may be picked up of contaminated pasture. The damage caused is due to irritation which interferes with feeding causing decreased weight gain, scratching result in wool loss, cuts and bruises. Sucking lice are also notorious for transmission of typhus and relapsing fever for humans (Sewell and Brockesby, 1990).

Sucking lice suck blood and can contribute to anemia as well as skin irritation (Kaufman, 2012). Lice bites are very irritating for the host skin. Sheep react scratching and rubbing intensively against objects and licking or biting the affected parts. This behavior can cause skin injuries susceptible of infection with secondary bacteria. Wool loss and reduced weight gain can be considerable. Lice infestations can also affect hide and leather quality resulting in reduced income. Milk production of dairy sheep and goat can also drop due to heavy lice infestations (Phillipson and Wright, 2011).

Both biting and sucking lice affect small ruminants. The important species in sheep and goats are found in the genus *Bovicola* and *Linognathus.* Most species of louse are highly host specific and many species specialize in infesting only one part of their host body and transfer to new hosts is by body contact, particularly under condition of close confinement (Peter, 1995).

Obviously, a critical factor in the likelihood of infestations beginning from non-sheep sources is the period for which lice can live away from sheep. Although the standard statement is that lice will not survive for more than 4 to 5 days after removal from sheep, it now seems that the potential period of survival is much longer than this. Laboratory studies were conducted to investigate the relative periods of survival of the different life stage of *Linognathus Ovillus* and the effects of temperature and the presence of wool on survival period. Large nymphs survived significantly longer than both small nymphs and adults and both age groups of nymphs survived longer than adults. This pattern was consistent across all temperatures. In a comparison of survival, at 4°C, 20°C, 25°C and 36.5°C lice consistently lived longest at 25°C and lice lived significantly longer at both 20°C and 36.5°C than at 4°C. The survival periods measured at 25°C (Crawford *et al.,* 2001).

Linognathus ovillus are susceptible to extremes in temperature and humidity and move up and down the wool fiber to accommodate these changes. They prefer to live at 37°C and 70-90% humidity. Above 39°C the number of eggs laid is reduced, and at 45°C no eggs are laid. On a hot day the fleece temperature on exposed parts of a sheep, with less than 25 mm of wool, may range from 45°C near the skin to 65°C at the wool tip. These temperatures are too hot for eggs and young lice to survive. Also lice and eggs do not survive extended periods of very low temperatures. Lice and their eggs do not survive for very long off the sheep. Survival of lice in wool on fences and in yards is very short. This is due to lack of food, exposure to sunlight and desiccation as well as temperature fluctuations between night and day (Levot, 2010).

When inspecting sheep for lice, at most times of the year greatest attention should be paid to the sides of the sheep. However, soon after shearing, inspections should also include the neck and lower body regions and areas where longer wool has been left. It should be noted that the chance of detecting lice in the early stages of an infestation is very low. For example, for a sheep with 10 lice, the probability of detecting the infestation by inspecting 10 fleece partings is less than 5%. Even with 40 partings the probability is less than 20% (James *et al*., 2002).

Biting lice feed on the skin and scurf. 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. Rubbing and/or bitting leads to wool loss, excoriations and serum exudation. The exuded serum from the wounds cause wool matting and the wound itself may attract blowflies. *B ovis* infestation in sheep is reported to cause an allergic dermatitis referred to as 'scatter cockle' (Pfeffer *et al*., 2010).

Lice infestations in small ruminants in Ethiopia were reported with an overall prevalence of 1.52% in goats and 2% in sheep (Haffeze, 2001) from central Ethiopia. The louse species identified were 0.8% *B. ovis* and 1.2% *Linognathus* species in sheep and 1.52 % *Linognathus* species in goats in central Ethiopia (Haffeze, 2001). However, results obtained from the examination of fresh sheep pelts showed a much higher infestation rate of 89.55% (Ermias, 2000).

The highest prevalence of lice were also those recently reported in sheep from Assela by Hailu (2010) who identified *Linognathus* spp. (75.5%) and *B. ovis* (67.1%) as well as Asnake *et al*. (2013) who recorded *Linognathus ovillus* (14.6%) and *B. ovis* (36.1%). Other reports were *B. ovis* infestation in 15.3% sheep and 27.9% goats and *L. ovillus* in 27.9% sheep in Tigray (Mulugeta *et al*., 2010) and *B. ovis* 26.64% in sheep in Wolayta Sodo (Yacob *et al*., 2008a). In the main, the lice species of sheep identified in studies conducted so far in Assela were *B. ovis* and *L. stenopsis* (Hailu, 2010).

2.1.2. Ticks and tick Infestation

Ticks are animals belonging to the phylum Arthropoda (Anderson and Magnarelli, 2008). Ticks are obligate, blood feeding ectoparasites of vertebrates, particularly mammals and birds (Anderson and Magnarelli, 2008). They belong to three families *Ixodiadae, Argasidae* and *Nutelielidae*. *Ixodidae*, known as hard ticks, contain almost all the species of ticks of veterinary importance (Mehlhorn and Armstrong, 2010). The second family *Argasidae,* known as soft ticks contains relatively small number of species of veterinary importance (Okello-onen *et al.,* 1999).

The body of tick comprises of two main regions: gnathosoma and idiosoma. Gnathosoma includes the basis capituli and mouthparts. The mouthparts consist of a pair of four-segmented palps, a pair of two-segmented chelicerae and a hypostome. Ticks use the chelicerate to penetrate the epidermis of their host and insert the hypostome with retrograde teeth into the wound. The retrograde teeth on the hypostome, together with the cement material secreted by tick's salivary glands, enhances attachment of tick to its host (Anderson and Magnarelli, 2008).

The life cycle of ticks vary widely. Some species pass their entire life on the host, others pass different stages of the life cycle on successive host, and others are parasitic only at the certain stages. Most ticks spend more time off the host, but are totally dependent on the host for sustenance. They are subjected to microenvironment condition when on the ground and thus tend to be more endemic in specific types of area. Ticks can exist for a long period of time without feeding (Peter, 1995).

Attachment to the host causes irritation of the skin, with subsequent ulceration and sometimes secondary bacterial infections. In addition, tick wounds may become infested by screw-worms or other agents of myiasis, and are also associated with the spread of bovine dermatophilosis caused by bacteria known as *Dermatophilosis congolensis.* Heavy infestations of ticks can result in anaemia, particularly in small animals, and the restlessness caused by the presence of large numbers of ticks can lead to a significant loss of weight and condition (de Castro, 1997). Ticks are important vectors for diseases like babesiosis, anaplasmosis and erlichiosis in domestic ruminants. They are known to exacerbate nonspecific disease symptoms like anemia, toxicosis and paralysis (CDC, 2015).

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, Haemaphysalis, Hyalomma* and *Rhipicephalus* (Minjauw and McLeod, 2003). Among these, *A. variegatum* and *Rh. decoloratus* are most important and widely distributed (Abebaw, 2004).

The tropical bont tick, *Amblyomma variegatum* Fabricius, is a three-host tick that originated in Africa. It has since spread to several countries, including the Caribbean islands, where it is known as the 'Senegalese tick' (CaribVet, 2011b) and the 'Antigua gold tick' (Pegram *et al*., 2004). The name 'Senegalese tick' came about because of the suspected introduction of the tick from cattle imports from Senegal to the Caribbean (Pegram *et al*., 2004). They are vividly colored and decorated ticks, especially the males (Merck, 2011).

Amblyomma variegatum, the ‘tropical bont tick’, are relatively large and have a bright coloration that makes them easily identifiable (CaribVet, 2011a). They sometimes have bright, yellow-gold coloration that is seen in the males that led to the common name, ‘Antigua gold tick’ (CFSPH, 2006). Females are usually brown and when fully engorged can be the size of a "nutmeg" (approximately 2 to 3 centimeters long) (CFSPH, 2006). As a member of the family Ixodidae, the tropical bont tick is considered a hard tick and has a scutum (Walker *et al*., 2003).

In females the scutum is smaller with a wide posterior angle and straight sides. Due to the scutum being smaller, it only provides partial coverage of the dorsal surface, which, as feeding or engorgement commences, covers a progressively smaller percentage of her body. The posterior lips of the female genital aperture forms a wide shaped “U”. In general, ‘tropical bont ticks’ also have long and thick mouthparts that allow them to become firmly embedded in their hosts (George *et al*., 2004). The ‘tropical bont tick’ has had a huge effect on the livestock industry, primarily through its transmission of heart water disease, *Ehrlichia ruminantium* (formerly *Cowdria ruminantium*) (OIE, 2009) and their association with dermatophilosis, *D. congolensis* (CaribVet, 2011b).

The tropical bont tick has also been implicated as a vector or potential vector for several diseases that include Crimean Congo haemorrhagic fever virus, Dugbe virus, yellow fever virus, *Rickettsia africae* (African tick bite fever) and *Jos virus* (Merck, 2011). Testing of ticks and seropositive blood tests of cattle have led to the conclusion that African tick bite fever is widespread in the islands, but there have been few positive human case reports (Kelly *et al*., 2010). There is a low incidence of documented reports of infection by other diseases in association with the tropical bont tick, and they occur primarily in Central Africa (Merck, 2011).

In Ethiopia, ticks occupy the first place amongst the external parasites by the economic loss they incurred when they infest livestock particularly cattle (Bekele, 2002). Ticks are common in all agro-ecological zones of Ethiopia. Extensive surveys have been carried out, in Ethiopia, on the distribution of tick on livestock in different region of the country. The overall prevalence of ticks infestation reported was 0.96% in sheep (Haffeze, 2001) from central Ethiopia; 5.27% in sheep (Molu, 2002) from southern range lands; 23.8% in sheep (Teshome, 2002) from Sidama zone; 66.5% in sheep (Zelalem, 1994) from Diredawa; and 0.1% in sheep (Tefera, 2004) from selected sites of Amhara region.

2.2. Ectoparasites Control Methods

Farmers mostly rely on the use of chemical acaricides and repellents to control ticks and limit the production losses. In order to reduce contact between ticks and vertebrate hosts, chemical repellents such as N,N-diethyl-*m*-toluamide (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 (Stafford and Kitron, 2002).

Organophosphates (diazinon, fampur, phosmet, and dichlorvos), synthetic pyrethroids (resmethrin, deltamethrin, and permethrin), carbamates (carbofuran, propoxur), growth regulator (fenoxycarb, methroprene), amitraz, fipronil and methandiol 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 (Foil *et al*., 2004). Moreover, evolved resistance to acaricides, which is a well-known problem with mosquitoes, is a persistent issue for tick species such as *Rhipicephallus microplus* that are chronically exposed by virtue of their close association with cattle to which the acaricides are applied (George *et al*., 2004).

2.3. Challenges of Ectoparasites Control

Problems posed by synthetic acaricides, resistant ticks are 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 decolouratus* (Mekonnen *et al.,* 2002). The resistance mechanism of ticks such as *R. microplus* to acaricides (coumaphos and diazinon) has been linked to an enhanced cytochrome P 450 monooxygenase-mediated detoxification (Liu *et al*., 2000).

Environmental pollution is a serious problem posed by the use of synthetic acaricides in tick control. Chemical compounds such as dichloro-diphenyl-trichloro-ethane (DDT), endosulfan and endosulfan sulphate are toxic and bioaccumulate in nature (Bhattacarya *et al*., 2003). Accumulation of these contaminants in water, soil and animals has been reported in Jamaica (Mansingh and Wilson, 1995). In 1961, the breeding number of peregrine falcons fell drastically and this was correlated with abnormally high residual levels of metabolites of DDT and dieldrin found in both the tissue and the carcasses of birds that fed on seeds treated with these compounds (Jarvis, 2000).

The accumulation of toxic chemicals obviously has an amplified effect on the food chain leading to magnification of toxic residues in animals occurring at higher levels of the food chain. Acaricides were also identified in honey bees using reversed-phase high performance liquid chromatography (Martel and Zeggane, 2002). It is obvious that such a situation is potentially dangerous to humans. Organophosphate accumulation in fatty tissue of mammals can lead to poisoning in man (Karalliede *et al*., 2003).

In Ethiopia also challenge of ectoparasites control are identified in which these parasites seriously damage sheep and goat skins, resulting in the rejection or downgrading of the skins. Export earnings from this important commodity are therefore drastically reduced. Even though, control programs started in few regions such as Amhara, Tigray and Oromia, still, reports indicate that these programs are far from recorded expected control and reduction of impact excreted by ectoparasites. Lack of awareness creation and absence of control on animal movement and poor quarantine policy might have resulted in ineffectiveness of the control campaigns (Yacob, 2014).

2.4. Alternative Approaches to Chemical Control

Ethno-veterinary plants use for tick control 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. Plant extract preparations are developed by farmers rather than scientists due to lack of finance to purchase synthetic acaricides which force them to depend on traditional methods of tick control that making them likely less toxic to the environment and non-targeted species (Castagnoli *et al*., 2002).

Due to the economic and medical importance of ticks, it is necessary to screen some ethno-veterinary plants that have acaricidal properties and could be used widely. Some of the advantages of promoting research on ethno-veterinary include the development of plant-derived chemicals which may be easily accessible by the rural communities and their low toxicity and biodegradability; thus, the need for their conservation. Plants are increasingly being recognized as possible sources of anti-tick agents. The use of plants or plant-based products for the control of arthropod ectoparasites on livestock is widespread among small scale livestock keepers in Africa (Matlebyane *et al*., 2010).

Furthermore, knowledge on traditional practices is orally transferred from one generation to another and often lacks scientific validation. A number of studies have so far been conducted to validate the use of plants for tick control. For instance, most recently Zorloni *et al*. (2010) demonstrated that extracts of *C. aurea* leaves used by the Borana people of northern Kenya and Southern Ethiopia to treat lice infestations in humans and calves. Besides, Magano *et al*. (2008) and Thembo *et al*. (2010) have described that *C. aurea* had anti-tick properties.

2.5. Herbs in Ethno-Veterinary Medicine

Knowledge can arise from scientific or traditional sources. Traditional knowledge has been described as a cumulative body of knowledge, practice and belief, evolving through adaptive processes and handed over through generations by cultural transmission (Berkes *et al.,* 2003). Traditional medicine is used throughout the world as it is heavily dependent on locally available plant species and plant-based products and capitalizes on traditional wisdom-repository of knowledge (Awas and Demissew, 2009). The wide spread use of traditional medicine could be attributed to cultural acceptability, economic affordability and efficacy against certain type of diseases as compared to modern medicines. Thus, different local communities in countries across the world have indigenous experience in various medicinal plants where they use their perceptions and experience to categorize plants and plant parts to be used when dealing with different ailments (Omoruyi *et al.,* 2012).

Ethno-veterinary medicine, the scientific term for traditional animal health care, encompasses the knowledge, skills, methods, practices, and beliefs about animal health care found among the members of a community (Nweze *et al*., 2004). Livestock owners have an excellent knowledge of ethno botany, which has formed the basis for screening plant materials as potential sources of medical drugs (Liu, 2004).

A plant part 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 (Doughari *et al.*, 2009).

Plants have played a central part in combating many ailments in human and livestock in many indigenous communities, including Africa (Busman *et al*., 2011). Traditional healers, and particularly medicinal plant herbalists, in Africa have a detailed knowledge-base of traditional medicine (Moshi *et al*., 2009), which is transferred orally from one generation to the next through professional healers, knowledgeable elders and/or ordinary people (Giday *et al*., 2007).

2. 5.1. Study plants

Plants have provided the basis for the traditional treatment of different types of diseases and still offer an enormous potential source of new chemotherapeutic agents. Different parts of plants have been used to treat ectoparasites both in animals and man. These are roots, barks, leaves and seeds (Bekele *et al*., 2012; Teklay *et al*., 2013). The following are plants selected for this study based on their traditional use by farming communities.

Calpurnia aurea (**Ait*****)* Benth.** Belongs to Fabaceae family and is commonly known as *Natal laburnum*. It is a small, multi-stemmed tree (3-4 m) tall. The leaves are about 15-25 cm long, each bearing 5-20 pairs of ovate to oblong leaflets, light green and 2-5 cm long, ending with a terminal one (Figure 1). The flowers are bright yellow, in racemes and the fruits are flat brownish pods (Zorloni, 2007). In southern Ethiopia, it is called ‘Cheka by the Borana people’. It is often found in overgrazed areas and is easily cultivated (Germishuizen and Meyer, 2003).

Figure 1: *Calpurnia aurea* (Ait.) Benth tree
Source (Tony, 2012)

Calpurnia aurea is widely distributed in Ethiopia. In Southern Ethiopia peoples soak leaves of *C. aurea* in cold water to treat louse infestations (pediculosis) in humans and calves. In Western Ethiopia, the juice of crushed leaves and bark is used for tick control (Regassa, 2000). In South-Western Ethiopia, the leaves of *C. aurea*, mixed with other plant species, are crushed and squeezed to obtain a juice, which is applied through the auricular route for 2 days to treat earache in humans. In the same area, the plant is traditionally used to treat rheumatism (Yineger *et al*., 2008). Antibacterial and antioxidant activity of *C. aurea* have been reported (Adedapo *et al*., 2008), and the plant has been used to treat bacterial dermatitis (Tadeg *et al*., 2005). It has also been used as a natural pesticide to improve grain storage (Blum and Bekele, 2002).

Calpurnia aurea leaves and powdered roots are used to destroy lice and to relieve itches. Unspecified parts are used to destroy maggots and the leaves are used to treat allergic rashes, particularly those caused by caterpillars. In East Africa, leaf sap is used to destroy maggots in wounds. In Nigeria, the seeds are used to treat abscesses. In Ethiopia, it is used to treat stomach complaints, headache, eye diseases, scabies and skin infection caused by ticks and as an insecticide as well (Asres *et al*., 2001).

Calpurnia aurea extracts are used in southern Ethiopia to protect stock against ticks. Acetone, hexane and water leaf extracts of *C. aurea* collected in southern Ethiopia were tested for repellent/attractant and acaricidal properties on unfed adult *R. pulchellus* ticks. In contrast to many other plant species evaluated, *C. aurea* extracts did not have repellent properties, but rather had a slight attractant capacity. With 20% and 10% acetone extracts, all ticks were either killed or their mobility severely compromised after 1 ml of extract was topically applied on the abdomen. The results proved the efficacy of the traditional use of this extract and may lead to a product that can be used commercially to protect animals against tick infestation, under subsistence as well as industrialized conditions (Zorloni, 2007).

***Ricinus communis* L.** (castor bean plant) belongs to the Euphorbiaceous family and is one of the medicinally important oil seed crop (Kumari *et al.*, 2008). In *in vitro* assays, extracts of *R. communis* and *Capparis spinosa* showed agglutinated activity and killed the parasites, *Leishmania major promastigotes*, and the aqueous leaf extracts showed excellent insecticidal activity against *Callosobruchus chinensis* (Upasani *et al*., 2003).

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 and Yasmin, 2011), antifungal (Shariff *et al*., 2006), antiviral and cytotoxicity (Sokmen, 2001), and insecticidal properties (Tounou *et al*., 2011).

The castor bean has been used to control insect pests in several crops. Aqueous castor-bean leaf extract has been shown to possess insecticidal activity against *Callosobruchus chinensis* (Coleoptera: *Bruchidae*) (Upasani *et al.,* 2003), *Cosmopolites sordidus* (Coleoptera: *Curculionidae*) (Tinzaara *et al.*, 2006), *Culex pipiens*, *Aedes caspius*, *Culiseta longiareolata*, and *Anopheles maculipennis* (Diptera: *Culicidae*) (Aouinty *et al.,* 2006); whereas the methanolic leaf extract had insecticidal activity against *C. chinensis* (Upasani *et al.*, 2003). In addition, both aqueous and acetone leaf extracts had different activity against *Acromyrmex lundi* (Himenoptera: *Formicidae*) (Caffarini *et al.,* 2008).

Castor oil showed insecticidal activity against *Zabrotes subfasciatus* (Coleoptera: Bruchidae) (Mushobozy *et al.,* 2009). Zahir *et al*. (2009) tested the acetone, chloroform, ethyl acetate, hexane, and methanol dried leaf and seed extracts of *R. communis* had been tested against the larvae of the cattle tick *R. (Boophilus) microplus*, sheep internal parasite *Paramphistomum cervi, f*ourth-instar larvae of *Anopheles subpictus Grassi* and *Culex tritaeniorhynchus*, and reported acaricidal and insecticidal activities against the adult of *Haemaphysalis bispinosa* and *Haemaphysalis maculata* (Zahir *et al.*, 2010). It also exhibited larvicidal and adult emergence inhibition activities against *Culex* quinquefasciatus, Anopheles stephensi, and Aedes albopictus (Mandal, 2010).

Figure 2: *Ricinus* communis L. tree

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

3. MATERIALS AND METHODS

3.1. Study Area

The laboratory experiment was conducted at Addis Ababa University College of Veterinary Medicine and Agriculture (CVMA), Parasitology laboratory at Bishoftu for louscidal activity test and Wollega University School of VeterinaryMedicine, parasitology laboratory where acaricidal activity using in vitro adult immersion test was carried out.

3.2. Study Design

Experimental study in which required number of unsexed adult tick and lice were assigned to treatment and control group with replication were done. *In vitro* acaricidal and louscidal efficacy of the extract of study plant on study parasite were evaluated.

3.3. Study Materials

3.3.1. Study parasites

Adult sheep lice: *Linognathus ovillus* were collected from naturally infested sheep bought from the Ada'a district of East Shoa zone. Coat brushing technique was used for collection of lice from sheep. The parasites were maintained in plastic cups into which water soaked cottons are placed to increase the humidity of the air found in the cups. The cups were covered by gauze to allow the free circulation of air into the cups and then the parasites were transported to CVMA, Parasitology laboratory. Identification of the parasites was conducted under a stereoscopic microscope according to the descriptions of Wall and Shearer (2001). Only, adult lice were used in these experiments.

Adult ticks: *Ambyomma variegatum* was collected from cattle for the *in vitro* acaricidal efficacy test from cattle brought to Diga Veterinary Clinic at Diga district of East Wollega zone. Ticks were collected from animals using forceps at main body sites namely: dewlap, brisket, belly and back, udder or scrotum, anogenital, and tail. Adult ticks collected from each of the main body sites were maintained in universal bottles separately and then transported to the Parasitology Laboratory of School of Veterinary Medicine, Wollega University for identification and *in vitro* efficacy test. Identification and recording of tick samples took place within a few hrs. of collection. Ticks were identified using stereomicroscope following the standard identification procedures described by Walker *et al*. (2003).

3.3.2 Study plant

The plants to be evaluated were selected and harvested from field according to literature and their usage in ethno-veterinary medicine in the country. *Calpurnia aurea* and *Ricinus communis* leaves were further investigated based on the results of efficacy of the crude extracts carried out previously (Askale, 2015; Sisay, 2015). Plant materials was collected from Wayu Tuka district, East Wollega zone of the Oromia region, located 331 km, west of Addis Ababa. This district is situated at an altitude between 1300 and 3140 meters above sea level. The annual temperature ranges from 12°C to 32°C and the average annual rainfall varies between 1250-1850mm (NMSA, 2014). The authenticity of the plant materials were confirmed by botanists at Aklilu Lemma Institute of Pathobiology (ALIPB), Addis Ababa, Ethiopia, where voucher specimens were deposited (collection numbers MA/001/06 and MA/002/06, respectively).

The Plant materials were dried in shade, at ambient temperature, pulverized, and milled to powder mechanically. The powdered materials were separately stored in dark tightly closed glass bottles at the Department of Pharmaceutical Chemistry and Pharmacognosy, School of Pharmacy, Addis Ababa University, for extraction. To reduce possible contamination, especially by fungi, latex gloves were worn when leaves were collected.

The Extracts were concentrated on a rotary evaporator under a reduced pressure of 22–26 mm Hg at 45°C and the residues obtained were weighed and stored at 4°C(Eloff *et al*., 2005). During preliminary screening, the adult tick and lice were used for bioassay test and experiments were conducted for 24 h at room temperature (37°C±2°C).

3.4. Plant Extraction

3.4.1. *Solvent-solvent fractionation of R. communis extracts*

The dried and powdered leaves (240 g) were exhaustively extracted successively with solvents of increasing polarity starting from petroleum ether (b.p. 40-60°C), chloroform, acetone, and methanol using Soxhlet apparatus (Rahuman *et al*., 2009). The extract was concentrated under reduced pressure at 40°C and the residue obtained was stored at 4°C.

3.4.2. *Extraction and isolation of alkaloids from C. aurea*

Dried powder leaves of *C. aurea* (500 g) were defatted with petroleum ether (2000 ml) by maceration and filtered. The marc was further macerated with 80% methanol for 72 hrs. Extraction was repeated 3x and filtered. The combined filtrate was dried under reduced pressure using Rota vapor to obtain a greenish semi-sold hydro alcoholic extract. This extract was taken in 2% (100 ml) of HCl to obtain an acidic solution (pH ~ 2), which was partitioned with equal volume of chloroform twice. The aqueous layer was basified with 10% ammonium hydroxide until a solution with a pH of 8-9 was obtained. The basic solution was extracted with equal volume of chloroform 3x. The organic layers were combined and evaporated under reduced pressure to yield a reddish brown semi-solid (Yubin *et al.,* 2014).

3.4.3. Analytical Thin Layer Chromatography (TLC)

Analytical TLC procedures utilized adsorption chromatography and were performed on silica gel 60 F_{254} precoated plates (0.2 mm) (E. Merck Darmstadt). The solvent system used for the alkaloids of *C. aurea* was a mixture of chloroform, methanol and 10% NH4OH in a ratio of 90:9:1 (v/v), while a mixture of chloroform, petroleum ether and ethyl acetate in a ratio of 8:2:2 was utilized for TLC analysis of the solvent extracts of *R. communis* (Sandam and Su, 2015). After air drying, the developed chromatograms were viewed under UV light. The alkaloids quench UV light of short wavelength (wavelength 254 nm) and appeared as dark or brown bands on the plates. Following this, the alkaloids were visualized by spraying with Dragendorff's reagent made up as follows: 0.8 g of bismuth sub nitrate in 50 ml of 20% v/v glacial acetic acid and 20 g of potassium iodide in 50 ml of distilled water were prepared separately and 5 ml of each of the solutions mixed with 90 ml of 22.2% v/v glacial acetic acid before use. The reagent gave orange-red color with all the alkaloids investigated (Asres *et al*., 1986).

Phytochemical Screening: The hydro alcoholic extracts of the leaves of *C. aurea* and *R. communis* were screened for the presence of secondary metabolites such as alkaloids, terpenoids, flavonoids, tannins, saponins, phenols and cardiac glycosides using standard qualitative phytochemical screening test procedures (Tiwari *et al*., 2011).

3.5. *In Vitro* Louscidal and Acaricidal Efficacy Tests

For the louscidal and acaricidal efficacy tests, the FAO modified protocol for adult immersion test was followed (FAO, 2004). The test substance were diluted in distilled water and 2% DMSO at the concentrations required for the bioassays. The test extracts were dissolved in distilled water and six concentrations were prepared arithmetically viz. 200, 100, 50, 25, 12.5 and 6.25 mg/ml were prepared by serial dilution. The *in vitro* tests were carried within 1hr after lice collection (Heukelbach *et al*., 2006).

Ten active lice and tick in three replications were placed in petri dishes and 2 ml of each concentration was directly added to the three replicate petri dishes and incubated at 27-28°C and 75-80% relative humidity for 24 hrs (Sanis *et al*., 2012). The tests were carried out on ten unsexed lice and ticks per replication were carried out (Nchu *et al*., 2005). These three replicates were treated with distilled water and 2% DMSO, as negative and using diazinon 60 EC as positive controls (Jadhav *et al*., 2007). The test solutions, positive (diazinon 60 EC) and negative (distilled water and 2% DMSO) controls were removed just after one and two minute contact time, using whatman No. 1 filter paper for lice and ticks respectively. The lice and tick in each petridish were closely observed for death under stereomicroscope at 30 min, 1hr, 2hr, 3hr, 6hr, 12hr and 24hr time intervals (Nanaa *et al*., 2010).

The criteria used for determination of death of lice and ticks were extremely strict. If any signs of life such as movement of antennae, gut cells or minimal legs movements were observed with stimulation by needle, the lice are categorized as alive. The criteria for death of ticks were determined by observing any minor signs of life such as minimal legs movement and phalengial reflexes with stimulation by forceps, categorize the parasites as alive. The lice and ticks were judged as dead, if there are no signs of movement at all (Jadhav *et al*., 2007).The percent mortality rate of lice was calculated as per Abbots formula cited by Krishnaveni and Venkatalakshmi (2014).

$$\text{Mortality \%} = \frac{\text{No. of mortality}}{\text{Total number of parasite}} \text{x100}$$

Mortality in the petri dishes treated with test substance was corrected to take account of control mortality using Abbott's correction. Classification of Louscidal and acaricidal effects are followed as previously used in *Melophagus ovinus* by Gemeda *et al*. (2014); as strong, mortality >80%; moderate, mortality 80–60%; weak, mortality 60–40%; little or no activity, mortality <40%.

3.6. Data Analysis

Collected raw data were stored in Microsoft Excel database system used for data management. SAS (r) Proprietary Software Version 9.00 (TS M0) was 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. The LC_{50} and LC_{90} value of the extract were determined applying regression equation analysis to the Probit transformed data of mortality using SPSS windows version 20. The P values <0.05 were regarded as significant.

4. RESULTS

4.1 Physicochemical Characteristics and Yield of Plant Extracts

The color, consistency, weights of powdered plant material, weight of dry extract, percentage yield and odor of the methanol, acetone, chloroform and petroleum extracts of *R. communis* and the alkaloid extract of *C. aurea* are recorded in Table 1. As shown in the table 1, among the four solvent fractions of *R. communis*, yield of the methanol extract was much more compared with all the other solvent extracts

Table 1: Physical characteristics and percent yield of the various solvent extracts of *Ricinus communis* and the alkaloid extract of *Calpurnia aurea*

Plant name	Local name afaan oromo/ Amharic	Method of extraction	Extraction solvent	Consistency	Colour	Weight of powder (g.)	Weight of extract (g)	Yield (%)
R. communis	Qobboo/Guloo	Soxhlet	Methanol	Sticky solid	Dark reddish	240	24	10
			Acetone	Sticky solid	Greyish black	240	8.2	3.4
			Chloroform	Semisolid	Dark greenish	240	8.6	3.6
			Petroleum ether	Sticky solid	Dark greenish	240	10	4.2
C. aurea	Ceekaa/Digita	Acid-base	HCL-NH_4OH	Semisolid	Reddish	500	2.5	0.5

4.1.1. *Phytochemical screening of solvent fractions of Ricinus communis leaf extract*

The successive extracts of petroleum ether, chloroform, acetone and methanol extracts were subjected to various chemical tests for the identification of their phytoconstituents. The results are shown in Table 2. Procedures followed for pytochemical screening of the solvent extracts of *R. communis* are shown in Appendex 2.

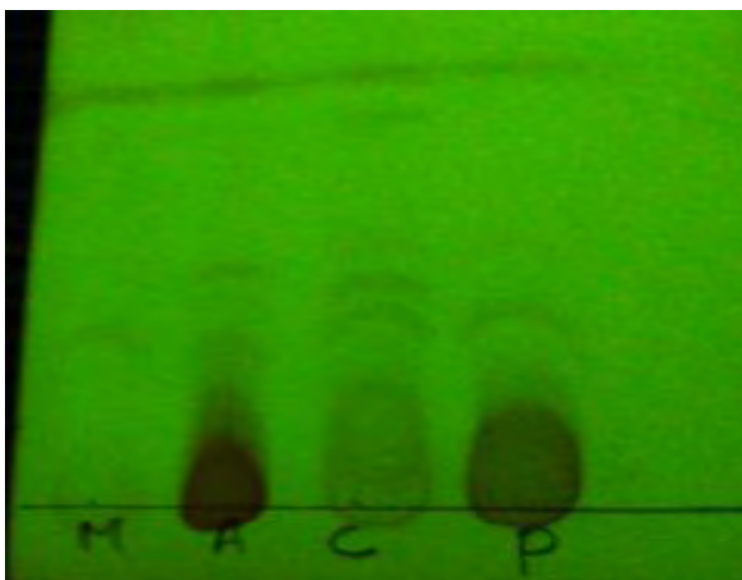
Table 2: Phytochemical screening results of the various solvent extracts of *Ricinus communis*

Secondary metabolites	Methanol extract	Acetone Extract	Chloroform extract	Petroleum ether
Saponin	+	+	+	-
Tanin	+	+	+	+
Phenolic	-	+	+	+
Steroids	-	+	+	+
Flavonoids	+	+	-	-
Phlobotanin	+	+	-	-
Glycosides	+	+	+	-
Triterpens	-	-	-	-
Alkaloids	-	-	+	+

Note: + indicate Prsence; - indicate absence;

4.1.2. *TLC of solvent fractions of Ricinus Communis leaf extract*

TLC of *R. communis* solvent extracts has been performed. As shown in Figure 3, the solvent system (Section 3.4.3) could not separate the components of the methanol extract, which remained on the baseline of the chromatogram. This is an indication that the compounds in the methanol extract are too polar for the relatively nonpolar solvent system to move them from the bottom of the plates.



- M=methanol, A=Acetone, C =chloroform, P=petroleum ether

Figure 3: TLC chromatogram of the various solvent extracts of *Ricinus communis*

[Adsorbent: Silica gel 60 F_{254} precoated plates (0.2 mm); Solvent system: Chloroform: Petroleum ether: Ethyl acetate (8:2:2); Visualization: UV light of short wavelength (254 nm); M: Methanol, A: Acetone, C: Chloroform, P: Petroleum ether].

4.1.3. *TLC of the alkaloid extract of Calpurnia aurea*

Examination of the alkaloid extract of *C. aurea* by analytical TLC using a mixture of chloroform, methanol and 10% NH4OH indicated the presence of at least three alkaloidal components (Figure 4). Although only three Dragendorff's positive bands were observed on the chromatogram, it is possible that the number of alkaloids could be much more if other solvent systems are used.

Figure 4: TLC chromatogram of the alkaloid extract of *Calpurnia aurea*

(Adsorbent: Silica gel 60 F_{254} precoated plates (0.2 mm); Solvent system: Chloroform: Methanol: 10% NH_4OH (90:9:1); Visualization: Dragendorff's spray reagent).

4.2. *In Vitro* Louscidal Activity of *Calpurnia aurea* and *Ricinus communis*

4.2.1 *In vitro louscidal activity of alkaloid extract of Calpurnia aurea*

The alkaloid extracts from leaf of *C. aurea* was tested for its louscidal activity against *Linognathus ovillus.* Percentage mortalities for the lice treated with the different concentrations of the *alkaloid* extract are shown in Table 3. The results showed that extract at concentrations of 200 mg/ml induced significantly (P <0.05) high levels of lice mortality compared to the reference drug diazinon at 30min respectively. After 1hr exposure no significance difference in all concentration and the reference drug diazinon (P>0.05).

The alkaloid extract at 200 and 100mg/ml concentration after 6hrs of exposure showed higher louscidal activity of 63.3% and 70% efficacy respectively. In addition, more than 50% of lice mortality was observed as early as 3 and 6hr after applying the alkaloid extract at the concentrations of 200 and 100 mg/ml, respectively. No mortality of Lice was found in the control group (treated with 2% DMSO). There was no statistically significant difference ($P > 0.05$) in the louscidal activity among the 100 and 200 mg/ml concentrations after 24hrs of exposure when compared to the reference drug (0.06% diazinon).

Table 3: Louscidal activity of different concentration of alkaloid extract of *Calpurnia aurea* on *Linognathus ovillus* at different times of exposure

Dose (mg/ml)	Mean mortality rate (%)±SE						
	30 min	**1hr**	**2hr**	**3hr**	**6hr**	**12hr**	**24hr**
200	20± 0.5^{aa}	33.3±0.3^{a}	46.6±0.3^{ba}	60±0.6^{ba}	70±0.5^{a}	86.6±0.8^{ba}	100±0.6^{ba}c
100	13.3 ± 0.3^{ba}	20±0.3^{a}	30±0.5^{ba}	40±0.3^{ba}	63.3±0.3^{a}	86.6±0.3^{a}	100±0.5^{bac}
50	6.6±0.3^{ba}	10±0.3^{a}	16.6±0.3^{ba}	26.6±0.3^{a}	53.3±0.3^{a}	63.3±0.3^{ba}	86.6±0.3^{ba}
25	3.3±0.3^{b}	6.6±0.3^{a}	20±0.3^{a}	26.6±0.0^{ba}	40±0.3^{a}	50±0.5^{ba}	70±0.3^{ba}
12.5	3.3±0.3^{b}	6.6±0.3^{a}	13.3±0.3^{ba}	23.3±0.0^{ba}	30±0.5^{a}	40±0.0^{ba}	53.3±0.5^{bac}
6.25	0±0.0^{b}	3.3±0.3^{a}	10±0.3^{ba}	13.3±0.3^{ba}	23.3±0.5^{a}	36.6±0.3b^{a}	43.3±0.3^{bc}
2% DMSO	0±0.0^{b}	0±0.0^{a}	0±0.0^{b}	0±0.0^{b}	0±0.3^{a}	0±0.0^{b}	0±0.0 ^{c}
Diazinon (0.06%)	3.3±0.33^{b}	10± 0.3^{a}	20±0.0^{ba}	23.3±0.3^{ba}	36.6±0.3^{a}	66.6±0.5^{a}	96.6±0.5^{a}

Values are expressed as mean of mortality % ± SE. Mortality % values with different superscripts within each column are significantly different ($P < 0.05$

4.2.2. *Evaluation of In vitro louscidal activity of acetone extracts of Ricinus communis*

Mortalities of lice treated with different concentration of the acetone fraction of *R. communis* are shown in Table 4. With exception of the 200 and 100mg/ml concentrations, the lower concentrations of the extract failed to show louscidal activity at 30min. There is no significant difference (P >0.05) at 200 and 100 mg/ml concentration after 30 min of exposure when compared to the positive control. This implies higher concentrations had comparable effect with the reference drugs. After 12hrs, higher mortality percentage up to 73.3% and 80% were recorded on lice exposed to 100 and 200 mg/ml concentrations, respectively. After 24hr time exposure 200 and 100mg/ml concentrations of acetone extract showed 100% and 93.3% lice mortality respectively.

After 30 min, the four lower concentrations (50, 25, 12.5 and 6.25 mg/ml) produced significantly (P < 0.05) low louscidal activity as compared to 200 mg/ml, concentrations. Moreover, the acetone extract had significantly ($P < 0.05$) lower louscidal activity at 30 min compared to positive control (0.1% diazinon). After 24hrs, Exposure, , 200 and 100 mg/ml concentration showed high mortality of lice with statistically insignificant difference ($P > 0.05$) when compared to the reference drugs, both killing 80±0.3 and 73.3±0.8 of lice within 12hrs, and 100±0.0% and 93.3±0.5% within 24hrs respectively. When compared to the reference drug (diazinon), the extract was found to have comparable effects against *L. ovillus* at 200, 100 mg/ml concentrations after 2hr of exposure.

4.2.3. *In vitro louscidal activity of methanol fraction of Ricinus communis*

Different concentrations of methanol fractionated extracts of *R. communis* leaf extract were evaluated for their louscidal properties against *L. ovillus*. The results showed that the three higher extract concentrations induced significantly (P<0.05) high levels of lice mortality as compared to three lower concentrations of extract. There is no significance ($P = 0.67$) difference between all concentration and reference drug diazinon from 2hr to 12hr time of exposure (Table 4).

No dead lice were found in the control group (treated with distilled water). In addition, more than 50% of lice mortality was observed as early as 12 and 24hrs exposure to the methanol fraction at concentrations of 200 and 100 mg/ml, respectively. Significant increase in lice mortality began at 1hr and 2hrs, post exposure with 200 and 100 mg/ml concentrations of methanol fraction. After 24hr post exposure, diazinon caused significantly ($P<0.05$) higher lice mortality than the methanol fraction at all concentrations except 200mg/ml.

Analysis of data revealed that there was significant difference ($P<0.05$) in lice mortality after 24hrs, at 200mg/ml concentrations of the extract and reference drug. No more significance difference was observed in all concentration at 1hr and 6hr, time exposure. Statistical analysis of the results revealed that there was significant difference ($P<0.5$) in lice mortality after 24hrs at all concentrations of extract. There was no significant difference in lice mortality at 30 minutes, 2, 3, 6 and 12hrs, at all concentration of the extract ($P>0.05$).

Table 4: Louscidal activity of different concentrations of the acetone and methanol fractions of *Ricinus communis* against *Linognathus ovillus* at different times of exposure

Plant extract / control	Dose (mg/ml)	Mean mortality rate (%)±SE						
		30 min	**1hr**	**2hr**	**3hr**	**6hr**	**12hr**	**24hr**
Acetone	200	13.3±0.3^{a}	23.3±0.0^{a}	26.6±0.3^{a}	36.6±0.5^{a}	53.3±0.0^{ba}	80±0.3^{a}	100±0.3^{a}
	100	10±0.0^{ba}	13±0.3^{a}	16.6±0.3^{a}	30±0.3^{a}	53.3±0.3^{a}	73.3±0.8^{a}	93.3±0.5^{a}
	50	0±0.3^{bc}	6.6±0.3^{a}	13.3±0.0^{a}	16.6±0.5^{a}	30±0.3^{bac}	60±0.3^{ba}	70±0.5^{a}
	25	0±0.0^{c}	3.3±0.3^{a}	6.6±0.3^{a}	13.3±0.3^{a}	23.3±0.3^{bac}	30±0.3^{ba}	43.3±0.3^{a}
	12.5	0±0.0^{c}	3.3±0.3^{a}	6.6±0.3^{a}	10±0.3^{a}	10±0.5^{bac}	16.6±0.0^{ba}	23.3±0.6^{a}
	6.25	0±0.0^{c}	0±0.3^{a}	0±0.3^{a}	0±0.3^{a}	0±0.0^{c}	6.6±0.0^{ba}	13.3±0.3^{a}
	Diaz (0.06%)	6.6±0.3^{bac}	16.6±0.0^{a}	20±0.3^{a}	30±0.5^{a}	50±0.5^{ba}	73.3±0.3^{a}	96.6±0.6^{a}
	Distilled water	0±0.0^{c}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.3^{bc}	0±0.0^{b}	0±0.0^{a}
Methanol	200	3.3±0.3^{a}	20±0.3^{a}	30±0.5^{a}	46.6±0.8^{a}	63.3±0.3^{a}	76.6±0.6^{a}	90±0.3^{bdc}
	100	0±0^{a}	6.6±0.3^{ba}	13.3±0.3^{a}	26.6±0.3^{a}	40±0.3^{a}	60±0.5^{a}	83.3±0.3^{ba}
	50	0±0^{a}	6.6±0.3^{ba}	13.3±0.3^{a}	20±0.6^{a}	36.6±0.3^{a}	53.3±0.3^{a}	76.6±0.3^{ba}
	25	0±0^{a}	6.6±0.3^{b}	10±0.3^{a}	16.6±0.3^{a}	30±0.6^{a}	33.3±0.3^{a}	53.3±0^{bac}
	12.5	0±0^{a}	3.3±0.3^{b}	6.6±0.3^{a}	10±0.3^{a}	16.6±0.3^{a}	26.6±0.5^{a}	33.3±0.3^{dc}
	6.25	0±0^{a}	0±0^{b}	0±0^{a}	3.3±0.3^{a}	6.6±0.3^{a}	13.3±0.3^{a}	16.6±0.3^{d}
	Diaz (0.06%)	6.6±0.3^{a}	16.6±0^{ba}	23.3±0.3^{a}	30±0.3^{a}	46.6±0.3^{a}	66.6±0.5^{a}	93.3±0.5^{a}
	Distilled water	0±0^{a}	0±0^{b}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{d}

*Values are expressed as mean of mortality % ± SE. Mortality % values with different superscripts within each column are significantly different ($P < 0.05$).

4.2.4. *In vitro louscidal activity of chloroform fraction of Ricinus communis*

All concentrations of chloroform fraction of *R. communis* showed no louscidal activity at 30 minutes. After 12hrs of exposure, higher mortality percentage up to 50% was recorded on lice exposed to 200 mg/ml concentration. All concentrations of chloroform fraction showed statically significant ($P < 0.05$) difference in their activity as compared to reference drug at 30 min (Table 5). After 24hrs exposure at 200 and 100 mg/ml concentrations showed mortality of 73.3±0.3% and 53.3±0.3% respectively, which was statistically insignificant difference ($P > 0.05$) when compared to the reference drugs (Table 5). On the other hand, lower concentrations (12.5 and 6.25 mg/ml) of the extract had little louscidal activity against *L. ovillus* after 24hrs of exposure.

The highest concentrations (200 and 100mg/ml) showed moderate efficacy of lice mortality up to 53.3 and 73.3% respectively (Figure 8). Even though there was statistically significant ($P<0.05$) difference compared to the untreated control (2% DMSO) all the concentrations of the extract showed no significant ($P<0.05$) difference in mortality of lice. But when compared to the untreated control (distilled water) all the concentrations of the extract showed high mortality of lice. On the other hand, all four lower concentrations of the extract have fallen in the low activity classification after 24hrs of exposure.

4.2.5. *In vitro louscidal activity of the petroleum ether fraction of Ricinus communis*

With the exception of the 200 mg/ml concentration of the petroleum ether fraction of *R. communis* which caused 60% mortality, the other doses exerted mortalities of less than 40%. After 6hrs exposure, 200 mg/ml of the extract caused higher mortality as compared to the lower concentrations (≤100mg/ml). The extract at all concentrations had less activity than the reference drugs. After 12 hrs, higher percentage mortality up to 40% and 33.3% were recorded on lice exposed to concentration of 100 and 200 mg/ml respectively.

Table 5: Louscidal activity of the different concentrations of petroleum ether and chloroform fractions of *Ricinus communis* against *Linognathus ovillus* at different times of exposure

Plant Extracts	Dose (mg/ml)	Mean mortality rate (%)±SE						
		30min	1hr	2hr	3hr	6hr	12hr	24hr
Petroleum ether	200	3.3±0.3^{a}	6.6±0.3^{a}	10±0.3^{a}	13.3±0.3^{a}	26.6±0.6^{a}	40±0.3^{a}	60±0.6^{a}
	100	3.3±0.3^{a}	3.3±0.0^{a}	6.6±0.3^{a}	20±0.3^{a}	23.3±0.3^{a}	33.3±0.6^{a}	43.3±0.0^{ba}
	50	3.3±0.3^{a}	3.3±0.0^{a}	6.6±0.3^{a}	16.6±0.0^{a}	20±0.3^{a}	23.3±0.3^{a}	30±0.3^{ba}
	25	3.3±0.3^{a}	3.3±0.0^{a}	3.3±0.0^{a}	6.6±0.3^{a}	10±0.3^{a}	16.6±0.7^{a}	23.3±0.3^{ba}
	12.5	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	3.3±0.3^{a}	6.6±0.3^{a}	10±0.3^{a}	16.6±0.3^{ba}
	6.25	0±0.0 ^{a}	0±0.0 ^{a}	0±0.0 ^{a}	3.3±0.3^{a}	3.3±0.0^{a}	6.6±0.3^{a}	10±0.3^{b}
	Diazinon (0.06%)	3.3±0.3^{a}	10±0.3^{a}	16.6±0.7^{a}	26.6±0.6^{a}	36.6±0.6^{a}	56.6±0.6^{a}	76.6±0^{a}
	2% DMSO	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.3^{b}
Chloroform	200	3.3±0.3^{b}	10±0.3^{a}	13.3±0.3^{a}	26.6±0.3^{a}	43.3±0.3^{a}	56.6±0.3^{a}	73.3±0.3^{ba}
	100	3.3±0.3^{b}	6.6±0.3^{a}	13.3±0.3^{a}	20±0.3^{a}	23.3±0.3^{a}	40±0.3^{a}	53.3±0.3^{ba}
	50	3.3±0.3^{b}	6.6±0.3^{a}	13.3±0.3^{a}	13.3±0.0^{a}	20±0.3^{a}	30±0.5^{a}	40±0.5^{ba}
	25	0±0.0^{b}	3.3±0.3^{a}	6.6±0.3^{a}	10±0.3^{a}	10±0.0^{a}	30±0.0^{a}	36.6±0.0^{ba}
	12.5	0±0.0^{b}	0±0.0^{a}	3.3±0.3^{a}	6.6±0.3^{a}	13.3±0.6^{a}	16.6±0.3^{a}	23.3±0.3^{ba}
	6.25	0±0.0^{b}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	3.3±0.3^{a}	10±0.3^{a}	13.3±0.3^{b}
	Diazinon (0.06%)	20±0.5^{a}	26.6±0.3^{a}	33.3±0.3^{a}	40±0.6^{a}	56.6±0.6^{a}	70±0.6^{a}	93. 3±0.3^{a}
	2% DMSO	0±0.0^{b}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.3^{a}	0±0.0^{b}

*Values are expressed as mean of mortality % ± SE. Mortality % values with different superscripts within each column are significantly different ($P < 0.05$)

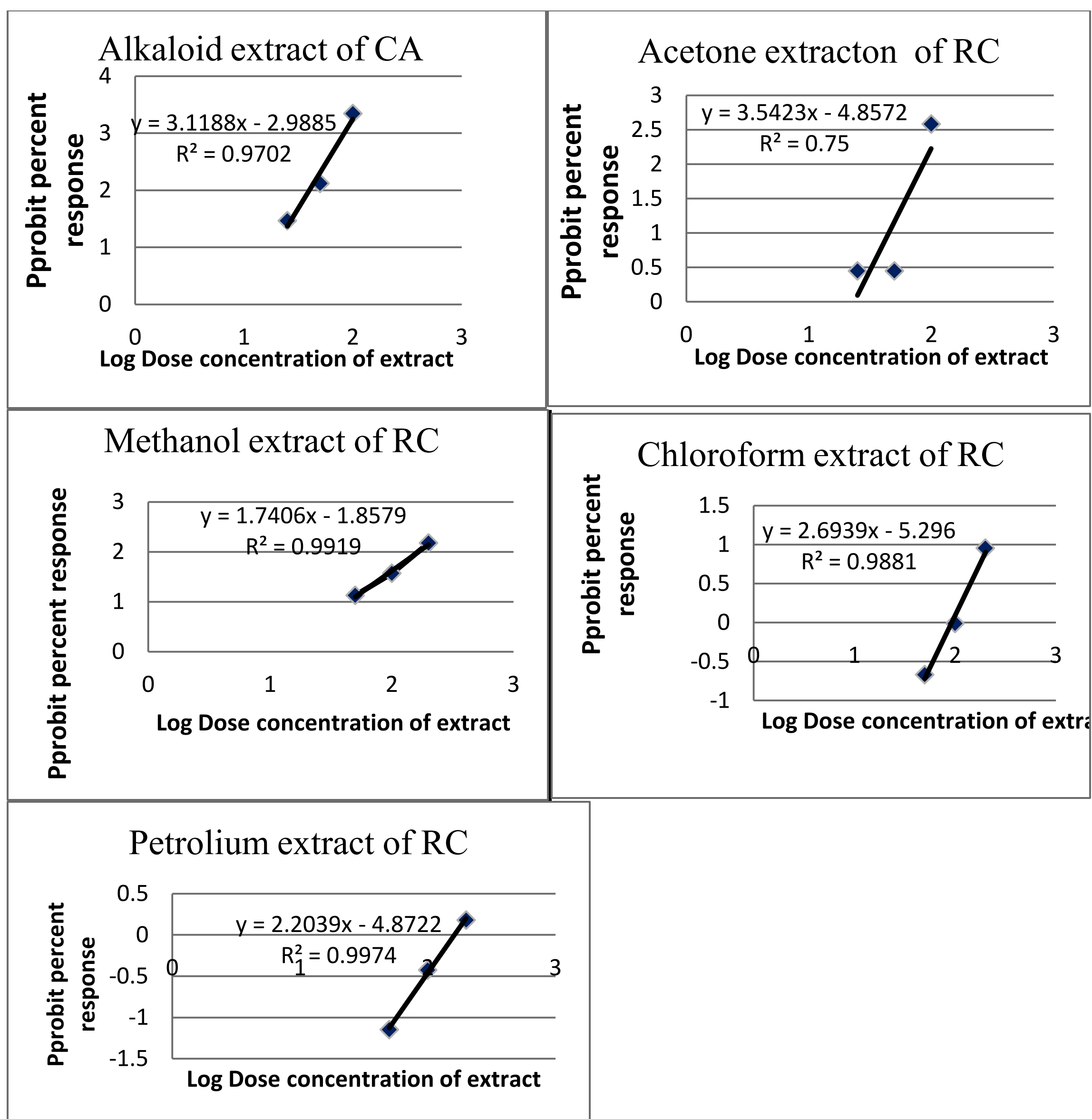


Figure 5: Log (Dose) - Probit Plot of *Linognathus ovillus* after 24hrs exposure to alkaloid extract of *Calpurnia aurea* and solvent fractions of *Ricinus communis*

Different concentrations of alkaloid of *C. aurea* and fraction of *Ricinus communis* extract depending on the time on *L. ovillus* were tested and regression equations were showed in (Figure 5). Dose response data of alkaloid *C. aurea* indicated the gradual increase in the mortality pattern with slope of 3.1188 and R^2 value of 0.9702 suggesting that 97.02% data were correlated with log concentration. Fractions of *Ricinus communis* extract dose response data also show gradual increases in mortality pattern with slope of 3.5423, 1.7406, 2.6939 and 2.2039 for acetone, methanol, and chloroform and petroleum ether, respectively.

4.3. *In vitro* Acaricidal Activity of *Calpurnia aurea* and *Ricinus communis*

4.3.1. *In vitro acaricidal activity of alkaloid extract of Calpurnia aurea on Amblyomma variegatum*

In vitro acaricidal activity of the Alkaloid extracts of *C. aurea* leaves was tested for its acaricidal activity against *A. variegatum.* Percentage mortalities for the ticks treated with the different concentrations of the alkaloid extract are shown in Table 6. The results showed that the alkaloid at 200 and 100 mg/ml concentrations induced high levels of tick mortality as compared to other concentration resulting in killing 96.6%, 76.6% of tick within 12hrs and 100%, 93.3% within 24hr (Table 6) respectively. The mortality of tick ranged from 46.6±0.00 to 100.00±0.33 at 24hr at 6.25 to 200 mg/ml concentrations, respectively. In addition, more than 50% of tick mortality was observed as early as 6hr upon exposure to the alkaloid extract at concentrations of 200, 100 and 50 mg/ml, respectively. When mortality was compared to the positive control, there were no statistically significant differences between the two higher concentrations ($P > 0.05$) after 6hr time exposure. When *A. variegatum* treated with all concentration of *C. aurea* alkaloid extract was compared to the negative controls (2% DMSO), there was statistically significant difference ($P<0.05$).

Table 6: Acaricidal activity of different concentrations of the alkaloid extract of *Calpurnia aurea* on *Amblyomma variegatum* at different times of exposure

Dose (mg/ml)	Mean mortality rate (%)±SE						
	30min	1hr	2hr	3hr	6hr	12hr	24hr
200	13.3±0.33[a]	20±0.33[ba]	36.6±0.33[a]	53.3±0.33[a]	73.3±0.58[a]	96.6±0.33[a]	100±0.33[ba]
100	6.6±0.33[a]	20±0.33[a]	33.3±0.67[a]	46.6±0.33[a]	60±0.67[a]	76.6±0.33[ba]	93.3±0.33[a]
50	3.3±0.33[a]	10±0.33[ba]	23.3±0.33[a]	33.3±0.33[a]	53.3±0.33[a]	66.6±0.33[ba]	83.3±0.33[a]
25	3.3±0.33[a]	10±0.33[ba]	13.3±0.33[a]	26.6±0.33[a]	43.3±0.33[a]	56.6±0.33[ba]	73.3±0.33[a]
12.5	0±0.00[a]	6.6±0.33[ba]	13.3±0.33[a]	23.3±0.58[a]	33.3±0.58[a]	46.6±0.33[ba]	63.3±0.33[a]
6.25	3.3±0.33[a]	3.3±0.33[ba]	10±0.33[a]	13.3±0.33[a]	26.6±0.58[a]	36.6±0.58[ba]	46.6±0.00[ba]
Diazinon 0.1%	6.6±0.33[a]	20±0.33[ba]	23.3±0.58[a]	30±0.58[a]	60±0.58[a]	83.3±0.33[a]	96.6±0.33[ba]
DMSO (2%)	0±0.00[a]	0±0.00[b]	0±0.00[a]	00±o.00[a]	0±0.00[a]	1±0.33[b]	3.3±33[b]

*Values are expressed as mean of mortality % ± SE. Mortality % values with different superscripts within each column are significantly different (P < 0.05).

4.3.2. *In vitro acaricidal activity of the acetone fraction of Ricinus communis extracts*

In vitro acaricidal activity of the acetone fraction *of R. communis* is depicted in Table 7. The extract at 200 and 100 mg/ml concentrations showed strong acaricidal activity of 93.3±0.5 % and 86.6±0.57%, respectively, after 24hr of exposure. In addition, more than 50% of tick mortality was observed as early as 6hr upon exposure of the ticks to the extract at concentrations of 200, and 100 mg/ml, respectively. With the exception of the extract at 200 mg/ml, all other concentrations failed to show strong acaricidal activity exposure for 3hrs.Analysis of the data revealed that there was no significant difference (P > 0.05) in tick mortality at 200 and100 mg/ml of extract and the positive control (0.1% Diazinon 60EC) in all time exposure. This shows that at all time interval these two higher concentration of extracts have comparable effect with that of the reference drug (0.1% Diazinon EC60). When *A. variegatum* treated with all concentration of the acetone fraction was compared to the negative controls (2% DMSO), there were statistically significant difference (P<0.05) (Table 7).

4.3.3. *In vitro acaricidal activity of the methanol fraction of Ricinus communis*

The acaricidal activity of the methanol fraction of *R. communis* on *A. variegatum* is depicted in Table 7. After 30 min of exposure time the extract at all concentrations and the reference drug (0.1% diazinon) exhibited a mortality of ≤6.6% indicating that both the extract and the reference require longer time to exert their action. 24hrs after exposure, mortalities of 90% and 80% were recorded for the 200 and 100 mg/ml concentrations of the extract, respectively. When compared to the positive control, there was no statistically significant difference ($P>0.05$) for the extracts at all concentrations from 30 min to 6hr time interval. However, the extract at a dose of 200 mg/ml and the reference drug displayed higher mortality of tick than the lower concentrations after 6hr time exposure. When *A. variegatum* treated with the methanol fraction of *R. communis* at all concentrations was compared to the negative controls (distilled water), there was statistically significant difference ($P<0.05$) in activity (Table 7).

Table 7: Acaricidal activity of the different concentration of acetone and methanol fractions of *Ricinus communis* against *Amblyomma variegatum* at different times of exposure

Plant Extracts / Control	**Dose (mg/ml)**	**Mean mortality rate (%)±SE**						
		30 min	**1hr**	**2hr**	**3hr**	**6hr**	**12hr**	**24hr**
	200	13.3 ± 0.33^{a}	20 ± 0.33^{a}	26.6 ± 0.33^{a}	33.3 ± 0.33^{a}	50 ± 0.88^{a}	73.3 ± 0.33^{a}	93.3 ± 0.57^{a}
	100	6.6 ± 0.33^{a}	16.6 ± 0.33^{a}	23.3 ± 0.57^{a}	43.3 ± 0.33^{a}	50 ± 0.57^{a}	66.6 ± 0.33^{ba}	86.6 ± 0.57^{a}
	50	6.6±0.33a	13.3 ± 0.33^{a}	23.3 ± 0.57^{a}	26.6 ± 0.33^{a}	43.3 ± 0.33^{a}	60 ± 0.66^{ba}	73.3 ± 0.66^{ba}
Acetone	25	3.3 ± 0.33^{a}	10 ± 0.33^{a}	20 ± 0.33^{a}	30 ± 0.57^{a}	43.3 ± 0.33^{a}	56.6 ± 0.33^{ba}	66.6 ± 0.00^{ba}
	12.51	0 ± 0.33^{a}	6.6 ± 0.66^{a}	13.3 ± 0.00^{a}	26.6 ± 0.33^{a}	33.3 ± 0.33^{a}	53.3 ± 0.57^{ba}	63.3 ± 0.00^{ba}
	6.25	0 ± 0.33^{a}	0 ± 0.33^{a}	10 ± 0.33^{a}	13.3 ± 0.33^{a}	26.6 ± 0.33^{a}	43.3 ± 0.33^{ba}	50 ± 0.33^{ba}
Diazinon (0.1%)		10 ± 0.57^{a}	13.3 ± 0.33^{a}	33.3 ± 0.00^{a}	40 ± 0.66^{a}	60 ± 0.57^{a}	80 ± 0.57^{ba}	96.6 ± 0.33^{ba}
Distilled water		0 ± 0.00^{a}	0 ± 0.00^{a}	0 ± 0.00^{a}	0 ± 0.00^{a}	0 ± 0.00^{a}	0 ± 0.00^{b}	0 ± 0.00^{b}
	200	6.6 ± 0.33^{a}	16.6 ± 0.00^{a}	30 ± 0.33^{a}	40 ± 1.00^{a}	53.3 ± 0.33^{ba}	73.3 ± 0.33^{a}	90 ± 0.33^{a}
	100	6.6 ± 0.33^{a}	16.6 ± 0.57^{a}	26.6 ± 0.57^{a}	36.6 ± 0.00^{a}	50 ± 0.33^{ba}	66.6 ± 0.33^{bac}	80 ± 0.66^{a}
	50	3.3 ± 0.33^{a}	10 ± 0.33^{a}	16.6 ± 0.33^{a}	23.3 ± 0.33^{a}	40 ± 0.33^{a}	56.6 ± 0.33^{bac}	70 ± 0.33^{a}
Methanol	25	3.3 ± 0.33^{a}	6.6 ± 0.33^{a}	13.3 ± 0.33^{a}	16.6 ± 0.33^{a}	30 ± 0.33^{ba}	40 ± 0.33^{bac}	50 ± 0.57^{a}
	12.5	3.3 ± 0.33^{a}	6.6 ± 0.33^{a}	13.3 ± 0.33^{a}	20 ± 0.66^{a}	30 ± 0.57^{ba}	36.6 ± 0.66^{bac}	46.6 ± 0.00^{a}
	6.25	0 ± 0.00^{a}	6.6 ± 0.33^{a}	10 ± 0.33^{a}	16.6 ± 0.33^{a}	23.3 ± 0.33^{ba}	26.6 ± 0.33^{bc}	40 ± 0.33^{a}
Diazinon (0.1%)		6.6 ± 0.33^{a}	16.6 ± 0.00^{a}	33.3 ± 0.33^{a}	36.6 ± 0.33^{a}	56.6 ± 0.00^{a}	76.6 ± 1.00^{ba}	93.3 ± 0.33^{a}
Distilled water (2%)		0 ± 0.00^{a}	0 ± 0.00^{a}	0 ± 0.00^{a}	0 ± 0.00^{a}	0 ± 0.00^{b}	0 ± 0.00^{c}	0 ± 0.00^{b}

*Values are expressed as mean of mortality%±SE. Mortality% values with different superscripts within each column are significantly different ($P < 0.05$).

4.3.4. *In vitro acaricidal activity of chloroform fraction of Ricinus communis*

With the exception of the 200 mg/ml concentration of the chloroform fraction of *R. communis* leaf extract, which showed low acaricidal activity, all the other fractions failed to exhibit any effect 30 min after exposure. However after 12 and 24hr exposure, a percentage mortality of 50% was recorded on *A. variegatum* by the highest dose of 200 mg/ml. After 24 hr exposure to doses of 200 and 100 mg/ml mortality rates of 56.6% and 50%, respectively, were recorded, which were no statistically significant ($P > 0.05$) when compared to the reference drugs (Table 8). On the other hand, lower concentrations (≤50 mg/ml) of the extract showed little acaricidal activity after 24hr of exposure.

All the doses of chloroform fraction showed no statically significant ($P > 0.05$) difference in their activity (Table 8). Moreover, this fraction had significantly ($P < 0.05$) lower acaricidal activity as compared to the positive control (0.1% diazinon 60EC). But when compared to the untreated control (distilled water) all the dose of the extract showed statistically significance difference ($p<0.05$) in causing mortality of tick. On the other hand, all four lower concentrations of the extract have fallen in the low activity category after 24hr of exposure (Table 8).

4.3.5. *In vitro acaricidal activity of petroleum ether fractions of Ricinus communis*

The activity of the Petroleum ether fraction of *Ricinus communis* on *Ambylomma variegatum* were shown at Table 8. Significant increase in tick mortality started 3hr post exposure with diazinon and 6hr post exposure with 200 mg/ml dose of the Petroleum ether extract. After 24hr post exposure period, diazinon has caused higher tick mortality than all the doses of extract. After 6hrs of exposures, 200 mg/ml of doses caused higher mortality than lower doses. There was no significant difference ($P>0.05$) at all concentration after 3hr of exposure when compared to the positive control. However all doses of the extract were less effective than the reference drug. After 12hrs of exposure, percentage mortalities of up to 40% and 30% were recorded for the 200 and 100 mg/ml doses, respectively.

After 24 hrs of exposure the 200 and 100 mg/ml doses of the acetone fraction showed 46.6% and 40% tick mortality, respectively, which were insignificant ($P > 0.05$) compared to other concentration. This fraction showed significantly ($P < 0.05$) lower acaricidal activity after 2hrs of exposure as compared to the positive control (0.1% diazinon). All doses of the extract showed statistically significance difference ($p<0.05$) in causing mortality of ticks as compared to the untreated control (2% DMSO).

Table 8: Acaricidal activity of different concentration of chloroform and petroleum fractionated extract of *Ricinus communis* against *Amblyomma variegatum* at different times of exposure

Plant Extracts /control	Dose (mg/ml)	Mean mortality rate (%)±SE						
		30min	**1hr**	**2hr**	**3hr**	**6hr**	**12hr**	**24hr**
chloroform	200	3.3±0.33[a]	6.6±0.33[a]	16.6±0.57[a]	23.3±0.33[a]	36.6±0.33[a]	50±0.33[a]	56.6±0.33[a]
	100	3.3±0.33[a]	6.6±0.33[a]	16.6±0.00[a]	20±0.33[a]	33.3±0.33[a]	40±0.33[a]	50±0.00[b]
	50	0±0.00[a]	6.6±0.33[a]	6.6±0.00[a]	16.6±0.57[a]	26.6±0.00[a]	36.6±0.00[a]	43.3±0.66[ba]
	25	0±0.00[a]	0±0.00[a]	6.6±0.33[a]	6.6±0.33[a]	10±0.33[a]	23.3±0.00[a]	26.6±0.33[ba]
	12.5	0±0.00[a]	3.3±0.33[a]	3.3±0.00[a]	10±0.33[a]	16.6±0.33[a]	20±0.33[a]	23.3±0.33[b]
	6.25	0±0.00[a]	0±0.00[a]	3.3±0.33[a]	6.6±0.33[a]	13.3±0.33[a]	13.3±0.00[a]	16.6±0.33[ba]
	Diazinon (0.1%)	6.6±0.33[a]	16.6±0.00[a]	23.3±0.33[a]	33.3±0.57[a]	46.6±0.33[a]	63.3±0.33[a]	90±0.33[a]
	2% DMSO	0±0.00[a]	0±0.00[a]	0±0.00[a]	0±0.00[a]	0±0.00a	0±0.00[a]	0±0.00[b]
Petroleum ether	200	3.3±0.33[a]	6.6±0.33[a]	10±0.33[a]	16.6±0.33[a]	26.6±0.57[a]	40±0.33[a]	46.6±0.33[ba]
	100	0±0.00[a]	3.3±0.33[a]	10±0.33[a]	13.3±0.33[a]	23.3±0.57[a]	30±0.33[a]	40±0.00[ba]
	501	0±0.33[a]	3.3±0.33[a]	6.6±0.33[a]	10±0.33[a]	16.6±0.33[a]	30±0.33[a]	36.6±0.33[ba]
	25	0±0.33[a]	0±0.33[a]	3.3±0.33[a]	3.3±0.00[a]	13.3±0.00[a]	26.6±0.33[a]	33.3±0.33[ba]
	12.5	0±0.33[a]	0±0.33[a]	3.3±0.33[a]	3.3±0.00[a]	10±0.33[a]	16.6±0.33[a]	30±0.33[ba]
	6.25	0±0.00[a]	0±0.00[a]	0±0.00[a]	3.3±0.33[a]	6.6±0.33[a]	16.6±0.00[a]	23.3±0.33[ba]
	Diazinon. (0.1%)	3.3±0.33[a]	13.3±0.00[a]	23.3±0.57[a]	33.3±0.57[a]	50±0.33[a]	66.6±0.66[a]	86.6±0.57[a]
	2% DMSO	0±0.00[a]	0±0.00[a]	0±0.00[a]	0±0.00[a]	0±0.00[a]	0±0.00[a]	0±0.00[b]

*Values are expressed as mean of mortality % ± SE. Mortality % values with different superscripts within each column significantly different ($P < 0.05$).

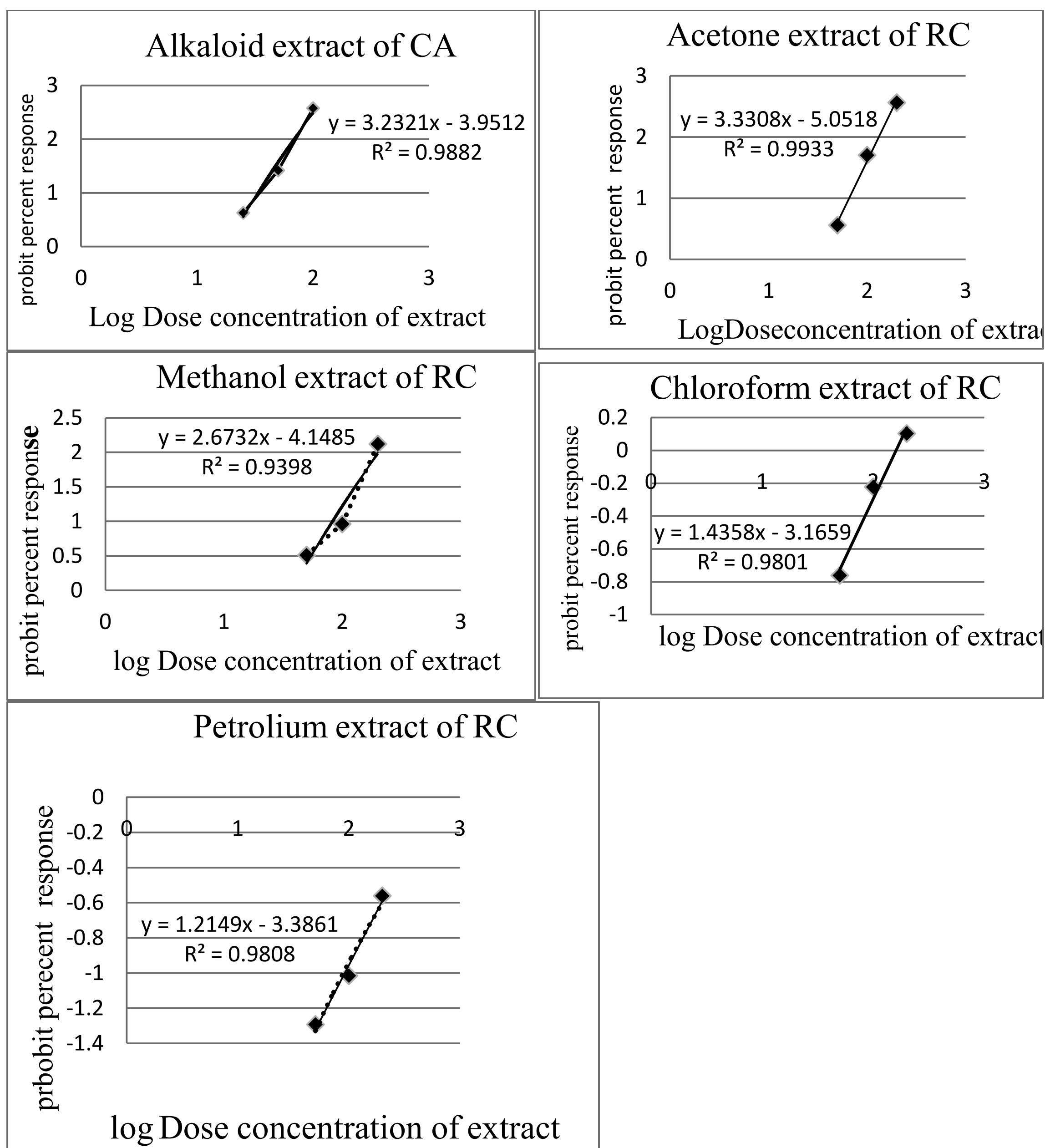


Figure 6: Log (Dose) - Probit Plot of *Amblyomma variegatum* exposed various dose of alkaloid extract *Calpurnia aurea* and solvent fractions of *Ricinus communis* after 24hr time exposure.

4.4.2. *Acaricidal and louscidal activity comparison between Calpurnia aurea* alkaloid and *solvent fractions of Ricinus communis*

Alkaloid extract of *C. aurea* and polar fractions *of R. communis* induced significant (P < 0.05) mortality (appendix) after 2hrs post exposure than chloroform and petroleum ether fraction of *R. communis*. Alkaloid extract cause high mortality than acetone and methanol which was significantly different from polar fraction with significance level of (P<0.05) respectively. After 24hr post exposure alkaloid and polar fraction(acetone and methanol) of *R. communis* cause significant mortality compared to non-polar(chloroform and petroleum ether) fractions of *R. communis* (Table 9).

Table 9: Comparison between extract effect of louscidal and acaricidal of alkaloid of *Calpurnia aurea* and fractions of *Ricinus communis*

Types of extracts		Significance level of parasite mortality after 24hrs					
		Lice			Tick		
		SE	95%CI	P Value	SE	95%CI	P Value
Alkaloid	Acetone	0.287	-0.65, 0.49)	0.772	0.242	-0.51, 0.45	0.893
	Methanol	0.287	-0.40, 0.74	0.563	0.242	-0.39, 0.57	0.703
	Chloroform	0.287	0.01, 1.15	0.045	0.242	-0.01, 0.95	0.056*
	Petroleum	0.291	0.01, 1.16	0.047	0.242	0.03, 0.99	0.038*
Acetone	Methanol	0.287	-0.32, 0.82	0.386	0.240	-0.35, 0.60	0.603
	Chloroform	0.287	0.10, 1.24	0.022	0.240	0.03, 0.97	0.039*
	Petroleum	0.291	0.09, 1.24	0.024	0.240	0.07, 1.02	0.026*
Methanol	Chloroform	0.287	-0.15, 0.99	0.150	0.240	-0.10, 0.85	0.120
	Petroleum	0.291	-0.16, 0.99	0.154	0.240	-0.06, 0.89	0.085
Chlorofor	Petroleum	0.291	-0.58,. 58	1.000	0.240	-0.43, 0.52	0.862

Note : SE=Standard error

After 24 hr post exposure, 200 and 100 mg/ml doses of the alkaloid extract caused lice mortality of 100±0.6% 100±0.5%, whereas the percentage mortality levels against ticks were 100±0.33 and 93.3±0.33, respectively. After 24 hrs, the LC_{50} and LC_{90} values (with 95% confidence limits) of the alkaloid extract for lice and ticks were estimated 9.08 mg/ml (6.21-13.47), 17.65 mg/ml (11.71-22.49) and 16.69 mg/ml (11.77, 26.64) and 31.69 mg/ml (21.25-50.72), respectively (Table 9). Based on the LC_{50} and LC_{90} values, the alkaloid extract was found to be more effective in killing of lice than tick (Table 10). As compared to the alkaloid extract of *C. aurea*, the solvent fractions of *R. communis* were less effective in both lice and ticks. Based on the calculated LC_{50} and LC_{90} values (Table 9), among the solvent fractions of *R. communis*, the less polar solvents extracts of chloroform and petroleum ether exhibited less louscidal and acaricidal effects than the more polar solvent extracts of acetone and methanol.

Table 10: LC_{50} and LC_{90} with 95 % CI of alkaloid extract of *Calpurnia aurea* and solvent fractions of *Ricinus communis* obtained by AIT against *Amblyomma variegatum* and *Linognathus ovillus* after 24hrs time exposure

Plant Extracts	**Test parasite**	**Regression equation**	R^2	LC_{50}**(mg/ml) (95%CI)**	$LC_{90(}$**mg/ml (95 % CI)**
Alkaloid	lice	y = 3.1188x - 2.9885	0.9702	9.08(6.21-13.47)	17.65(11.71-22.49)
	tick	y = 3.2321x - 3.9512	0.9882	16.69(11.77-26.64)	31.69(21.25-50.72)
Acetone	lice	y = 3.5423x - 4.8572	0.9612	23.50(14.93-31.20)	42.19(31.91-77.65)
	tick	y = 3.3308x - 5.0518	0.9933	32.86(21.82-74.56)	61.69(33.14-145.41)
methanol	lice	y = 1.7406x - 1.8579	0.9919	11.67(5.90-14.80)	38.41(29.42-57.57)
	tick	y = 2.6732x - 4.1485	0.9398	35.63(23.35-66.77)	77.11(45.75-179.89)
chloroform	lice	y = 2.6939x - 5.296	0.9881	92.45(60.54-192.31)	199.53(123.00-675.00)
	tick	y = 1.4358x - 3.1659	0.9801	160.32(84.31-824.89)	678.89(279.39-1853.29)
Petroleum ether	lice	y = 2.2039x - 4.8722	0.9974	162.44(86.12-467.20)	415.99(178.71-2236.74)
	tick	y = 1.2149x - 3.3861	0.980	612.41(137-1747)	3372.44(590-15057)

*Tick=Amblyomma variegatum, lice=Linognathus ovillus,

5. DISCUSSION

Extraction of the leaves of *R. communis* with solvents of varying polarity yielded similar results (Table 1) with those reported by Singh *et al*. (2010). However, there were differences in the yields of the petroleum ether and methanol extracts. Differences in percentage yields of extracts are not uncommon since the method of extraction and conditions of environment in which the plant grows, such as climate and altitude affect yields. In support of this, Perucci *et al.* (1995) described that solubility of various plant ingredients depends on the extraction methods. In the present study the yield of the methanol extract was much higher than those obtained by using the other relatively less polar solvents. This is consistent with the results reported for yields of other plant species (Masoko *et al.,* 2005).

Phytochemical screening of the various solvent extracts of *R. communis* leaves revealed that the plant contains saponins, tannins, phenolic compounds, steroids, flavonoids, glycosides and alkaloids (Table 2). The current findings are similar to those reported previously by Singh *et al.* (2010), but different with the findings of Kumar *et al*.(2011), who reported the absence of alkaloids in leaf extracts of *R. communis*. Since the types and quantities of secondary metabolites derived from natural sources depend on growth conditions of the plants, it is not surprising that they are subject to variability (Solomon *et al*., 2013).

Similarly, extraction of alkaloids from the 80% methanolic extract of *C. aurea* leaves yielded 0.5% (w/w) of alkaloids. Previous report by Asres *et al*. (1986) indicated that the hydro alcoholic extract of *C. aurea* leaves contain 1.65% (w/w) alkaloids. The variability in the yield of alkaloids could be attributed to the time of collection which is among the main factors that affect alkaloid contents in plants. Although the same authors isolated 13 quinolizidine alkaloids, only two of the alkaloids constituted 90% of the alkaloidal content of the plant. In the present study, TLC examination of the crude alkaloid extract of *C. aurea* leaves furnished three major bands that may contain more than three alkaloids.

Investigation of the alkaloid extract of *C. aurea* leaves for its louscidal and acaricidal effects against *L. ovillus* and *A. variegatum*, respectively, revealed that it possesses potent activities against both ectoparasites. At higher doses of 200,100 and 50 mg/ml, the extract showed comparable louscidal and acaricidal activities to the standard drug diazinon. To the best of our knowledge, no report exists in the literature concerning the louscidal activity of the alkaloids of *C. aurea* against *L. ovillus*, although Silva-Aguayo (2006) has documented their insecticidal properties. In a previous study, it has been reported that that the insecticidal properties of the some plant extracts could be due to the involvement of anticholinergic alkaloids such as scopolamine, hyoscyamine, meteloidine, and atropine (Berkov *et al*., 2006; Aronson, 2009). As *tropane* alkaloids have not been isolated from *C. aurea*, *quinolizidine* alkaloids could be regarded as another class of alkaloids that possess insecticidal properties.

Comparative studies on the acaricidal and louscidal effects of the alkaloids of *C. aurea* against *L. ovillus* and *A. variegatum* indicated that the alkaloids are more active on lice than ticks. Moreover, from the calculated LC_{90} values after 24hr time exposure (Table 10), it is clearly seen that the effect of the alkaloids against lice was in a dose-dependent manner i.e. percentage mortality increased with an increase in the dose of the extract. This is in line with Levetin and McMahon (2003), who reported that difference between a medicinal and a toxic effect of many alkaloids (or any drug) is often a matter of dosage.

Acaricidal and louscidal activities of the alkaloid extract of *C. aurea* against *L. ovillus* and *A. variegatum* illustrate that correlations exist between the popular ancestral perception and genuine antiparasitic activities. Moreover, it lends support to further studies aimed at the isolation and identification of alkaloid(s) with better therapeutic value

The solvent fractions of the leaf extracts of *R. communis* demonstrated varying acaricidal and louscidal effects against *L. ovillus* and *A. variegatum*. Whilst the relatively non polar solvent extracts (petroleum ether and chloroform) displayed weak activities, the more polar solvent extracts of acetone and methanol were highly active. At doses of ≥50mg/ml, the polar solvent extracts exhibited strong louscidal activity which was equivalent to the commercial acaricides, diazinon.

Although effects of the organic solvent extracts of *R. communis* against *L. ovillus* have not been reported prior to this study, the plant is used to control insect pests in several crops. For example, the aqueous leaf extract *R. communis* has been shown to possess insecticidal activity against *Callosobruchus chinensis* (Coleoptera: *Bruchida*e) (Upasani *et al*., 2003), and *Anopheles maculipennis* (Diptera: *Culicidae*) (Aouinty *et al.*, 2006). Similarly the methanolic leaf extract of the same plant showed insecticidal activity against *C. chinensis* (Upasani *et al.*, 2003). It has been reported that the effects of such medicinal plants arise as a result of various secondary metabolites which are present in these plants (Edeoga *et al.*, 2005). Similar to our current results which demonstrated variations in activity among the different solvent fractions, previous findings showed that the New World ant *Acromyrmex lundi* (Himenoptera: *Formicidae*) has varying susceptibility to the aqueous and acetone leaf extracts of *R. communis* (Caffarini et al., 2008).

In the present work, the various solvent fractions of the leaf extract of *R. communis* have been shown to exhibit activity against *A. variegatum* particularly at higher concentrations after 24hrs of exposure. To date, there appears to have been no published scientific report on the effect of the extract of *R. communis* against A. variegatum. However, the acaricidal effect of this plant against the larvae of *Boophilus microplus* has been reported (Zahir *et al.*, 2009). Zahir *et al*. (2010) further showed mortality of *Haemaphysalis bispinosa* in the range of 77.0 ± 2.1–100.0 ± 0.0% when treated with *R. communis* leaf extracts prepared in various organic solvents.

Other reports indicate that *R. communis* extracts exhibit cidal effect against ticks (Ghosh *et al.*, 2015). Although seed extracts of *R. communis* are known to be highly toxic due to the presence of the glycoprotein ricin(Kozlov *et al.*, 2006; Tounou *et al.*, 2011), this most poisonous natural product has been shown to occur in the leaves of the plant in a very low concentration (El-Nikhely *et al*.,2007). Moreover, the organic solvent extracts of the leaves of *R. communis* extracts are not expected to extract *ricin* due to its highly polar nature.
The current study also demonstrated that the acetone and methanol fractions of *R. communis* have a lethal effect (≥90±0.33%) against *A. variegatum*. After 24hrs of exposure, their LC_{50} values were found to be 32.86 and 35.63 mg/ml, respectively. On the other hand the effects of the chloroform and petroleum ether extracts were rather weak causing ≤56.6±0.33% death of the parasites. The LC_{50} values of these extracts were calculated to be 160.32 and 612.41 mg/ml, respectively, after 24hrs of exposure.

On the basis of the results of the current study, it can be concluded that the acaricidal and louscidal components of *R. communis* leaf extracts mainly reside in the polar solvents, although a low percentage of the active principle(s) appears to be present in the non-polar solvents. It is also possible that the active principles in the nonpolar solvents may differ from those that occur in the polar solvent. Although both the alkaloid extract of *C. aurea* and the various solvent fractions of *R. communis* were found to be lethal to *L. ovillus* and *A. Varigatum*, they appear to be more active against the former than the latter. This might be attributed to thickness of the integument in ticks as compared to lice, which may interfere with the absorption of the active ingredients present in the plant extracts. However, the mechanism(s) by which the plant extracts exert their action must be studied in order to know their actual mode of action

6. CONCLUSION AND RECOMMENDATIONS

The present study has demonstrated the *in vitro* acaricidal and louscidal activities of the alkaloids of *Calpurnia aurea* and various organic solvent fractions of *Ricinus communis* against *Linognathus ovillus* and *Ambylomma variegatum*. The findings further suggested that the active ingredients in the leaf extract of *Ricinus communis* reside in the polar fractions indicating that the active principles are Polar in nature. The current study showed that the alkaloids of *Calpurnia aurea* and, the acetone and methanol fractions of *Ricinus communis* possess strong activity which was comparable to that of the reference drug diazinon. There is a high possibility that these plant extracts provide effective eco-friendly herbal formulations for the control of lice and tick infestation on animals.

In line with above concluding remarks, the following recommendations are forwarded:

- Phytochemical investigations of the active extracts must be carried out in order to isolate and elucidate the structure(s) of the active constituents;
- Acute and chronic toxicity studies should be done in order to assess the safety of the extracts under field conditions;
- Further louscidal and acaricidal activity tests on other ectoparasites need to be done to know if the plants have broad spectrum of activity.

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8. APPENDICES

Appendix 1: TLC procedure followed during *Ricinus communis* extract

- **From each extract 0.1ml spotted on the TLC plate using capillary tube**
- TLC plate was kept in developing chamber which was prepared as 8:2:2 of chloroform, petroleum ether and ethyl acetate
- Wait till the mobile phase run up to distance of 10cm following chromatography
- The plate was dried gently using dryer
- The number of band was observed under UV light

Appendix 2: Preliminary phytochemical screening of solvent extracts

The extracts will be tested for the presence of bioactive compounds by using the following standard methods.

Test for Alkaloids: Crude extract will be mixed with 2ml of 1% HCl and heated gently. Mayer's reagent will be added to the mixture. Turbidity of the resulting precipitate will be taken as an evidence for the presence of alkaloids

Test for Flavonoids: Crude extract will be mixed with 2ml of 2% solution of NaOH. An intense yellow color will be formed which turned colorless on addition of few drops of dilute acid which indicated the presence of flavonoids

Test for Glycosides: 2ml of concentrated H_2SO_4 will be added carefully and shaken gently. A reddish brown color indicated the presence of steroidal ring. i.e. glycone portion of the glycoside

Test for Phenols: Crude extract will be mixed with 2ml of 2% solution of $FeCl_3$. A blue green or black coloration indicated the presence of phenols

Test for Proteins: When crude extract will be boiled with 2ml of 0.2% solution of Ninhydrin, violet color appeared suggesting the presence of proteins

Test for Saponins: Crude extract will be mixed with 5ml distilled water in a test tube and it will be shaken vigorously. The formation of stable foam will be taken as an indication for the presence of saponins

Test for Steroids: Crude extract will be mixed with 2ml of chloroform and concentrated H_2SO_4 will be added sidewise. A red color produced in the lower chloroform layer indicated the presence of steroids

Test for Tannins: Crude extract will be mixed with 2ml of 2% solution of $FeCl_3$. A blue – green or black coloration indicated the presence of tannins

Test for Terpenoids: Crude extract will be dissolved in 2ml of chloroform and evaporated to dryness. To this 2ml of concentrated H_2SO_4 will be added and heated for about 2 minutes

Source (Tiwari *et al*., 2011).

Appendix 3: Picture taken during plant collection and drying of Study plant

***Ricinus communis* (Qobbo)** ***Calpurnia aurea* (Ceekaa)**

Shade drying of study materials

Appendix 4: Picture of some of extraction equipment used for plant extraction

Soxhlet extractor **Rota vapor** **Sanction pump**

Separatory funnel

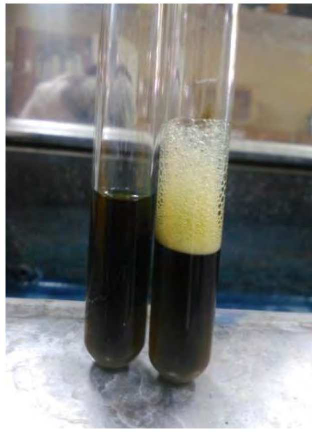
TLC Plate along with Developing chamber

Glass flask used in maceration

Appendix 5: Picture of some phytochemical screening of *Ricinus communis* extracts

A **B** **C**

A=Acetone fraction (Phenols) **B=methanol fraction (saponins)** **C=chloroform fraction (tannins)**



D **E** **F**

D=Saponins in chloroform fraction **E= methanol fraction (phenols)** **F= pet. Ether (alkaloid)**

Appendix 6: Picture taken during collection and bioassay of parasite

During tick collection from Calf **Adult tick immersed** **Died tick among immersed**

Lice infested sheep used in study **Dead lice among immersed in extract**

Appendix 7: In vitro louscidal and acaricidal efficacy 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
	200mg/ml									
	100mg/ml									
	50mg/ml									
	25mg/ml									
	12.5mg/ml									
	6.25mg/ml									
+ve control	0.1%/0.06% Diazinon									
Negative control	DMSO 2%									
	Distilled									

	water									