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AGRICULTURE**

DEPARTMENT OF BIOMEDICAL SCIENCE

MSC THESIS ON

**INVITRO ACARICIDAL ACTIVITY OF SELECTED MEDICINAL PLANTS AGAINST
TICKS AFFECTING DOMESTIC ANIMALS IN AND AROUND BISHOFTU**

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LIST OF APPREVIATIONS AND ACRONYMS

AIT	Adult Immersion Test
ALIPB	Aklilu Lemma Institute of Pathobiology
ANOVA	Analysis of Variance
CI	Confident Interval
CSA	Central Statistical Agency
DMSO	dimethyl sulfoxides
LC50	Median Lethal Concentration
LC99	Lethal Concentration that kill 99% of test population
LPT	Larval Packet Test
m. a. s. l	Meter Above Sea Level
ppm	Parts Per Million
Rota- Vapor RE	Rotary Evaporator
Spp	Species (plural)
TBDs	Tick Borne Diseases
USP	United state pharmacopeia
w/v	weight per volume

ABSTRACT

Ticks are among the most important ectoparasites affecting livestock in Ethiopia, causing economic losses through blood loss, skin damage, reduced productivity, and transmission of disease. Synthetic acaricides are commonly used for tick control, their effectiveness is threatened by acaricide resistance, environmental contamination, and residue concerns. Therefore, present study was carried out to evaluate the invitro acaricidal activity of crude methanolic and n-hexane extracts of five medicinal plants in central Ethiopia namely *Calpurnia aurea*, *Ricinus communis*, *Premna schimperi*, *Lepidium sativam*, and *Aloe yavellana* against adult *Amblyomma variegatum*, and *Rhipicephalus decoloratus* using invitro adult immersion test. Adult tick were collected from naturally infested cattle in and around Bishoftu. Five graded concentration crude extracts by both solvents 6.25, 12.5, 25, 50, and 100mg/mL were tested in triplicate at different time intervals (0.5, 1, 2, 3, 6, 12, and 24 hours). The tick mortality was recorded at each time interval. Diazinon 0.1%, and distilled water with tween 80 and DMSO were used as positive and negative control respectively. The phytochemical screening of five plant species showed that methanolic extract contained Alkaloids, Flavonoids, Terpenoids, Saponins, Glycoside, and Steroids, while n-hexane extracts contained only terpenoids, and steroids. All tested plants demonstrate significant dose and time dependent Acaricidal activity against adult ticks, with methanolic extracts proving most effective. Notably, at 100mg/mL after 24 hours, methanolic extract of *C. aurea* (90% mortality), and *P. schimperi* (83.3% mortality) matching or exceeding diazinon (80%). Based on LC50 values the methanolic extract of *P. schimperi* exhibited the highest Acaricidal potency (2.18mg/mL), followed by *R. communis* (3.84 mg/mL), *C. aurea* (6.79 mg/mL), *L. sativam* (8.58 mg/mL), and *A. yavellana* (20.17mg/mL). Overall, *P. schimperi*, and *C. aurea* methanolic extracts were showed promising as eco-friendly botanical acaricides against adult ticks, though further research is required to isolate their active compounds and validate their safety and efficacy under field condition.

Key words: *Acaricidal Activity; Central Ethiopia; Invitro Assay; Medicinal Plants; Tick Control*

1. INTRODUCTION

Ethiopia has the most significant livestock population in Africa. This livestock sector has been contributing considerable portion to the economy of the country and play an important role in providing export commodities such as live animal, hide, and skins to earn foreign exchange to the country (CSA 2017). However livestock production in many parts of the world is constrained by several factor (Onu and Shiferaw, 2013). Among the various constraints encountered, ectoparasites contribute to a wide range productivity problem (Nejash, 2016). Ectoparasites are very common and widely distributed in all agro-ecological zones of the world. approximately 1.49 billion cattle worldwide are susceptible to infestation with diverse ectoparasites fauna (León *et al.*, 2020). In Ethiopia hard tick from the genera *Rhipicephalus* or *Boophilus*, *Amblyomma*, and *Hyalomma* are widely distributed and are caused direct and indirect loss in livestock production (Girma *et al.*, 2024). Direct impact of tick infestation include blood loss, irritation, reduced weight gain, lowered milk yield, hide damage and skin rejection. Indirectly ticks affect cattle by serving as vector for pathogen and disease transmission (Kemal *et al.*, 2016; Nejash, 2016,).

The challenges of using chemical for tick controlling method are resistance, environmental toxicity, non-target impact, and impact of the environment on the efficacy of chemicals (Makwarela *et al.*, 2025). Due to this challenges many countries focused on new strategies of controlling ticks to overcome the problem associated with the use synthetic drugs. The new alternative control strategy could be phytotherapy, an important components of ethnoveterinary medicine (Madzimure *et al.*, 2011). Researches focused on the use of natural product from the botanical source used as acaricides, principally to minimize the increasing frequency of acaricide-resistant strain of tick. In Ethiopia farmer, pastoral and small holder livestock system, traditional botanical and ethnoveterinary tick control method are widely used instead of synthetic chemical acaricides due to accessibility, cost, and resistance (Nchu *et al.*, 2012).

Traditional medicinal plants are commonly used for tick control in Ethiopia and the increasing interest in botanical acaricides, however there is a limitation or fragmentation on the scientific evidence regarding their efficacy. The previous researches have commonly focused on individual plant species, different tick species, and single extraction method which make difficult to compare the acaricidal potential between them under standardized experimental condition. Additionally,

quantitative data on the acaricidal efficacy of two plant (*P. schimperi*, and *L. sativum*) selected for present studies are particularly scarce despite their frequent use by the society. The comparative information on effectiveness of methanol, and n-hexane extracts of selected medicinal plants against tick species in Ethiopia is lacking. Therefore, the present work was aimed to generate scientific evidence on the acaricidal efficacy of methanol and n-hexane extract of the five medicinal plants traditionally used by livestock farmers and owners, and to identify the promising candidates for the development of environmentally friendly and sustainable alternatives to synthetic chemical acaricides used for tick control in central Ethiopia.

The specific objectives are:-

- To determine the dose – and time -dependent acaricidal activity of methanolic and n-hexane extracts of five plant species against adult ticks.
- To compare the acaricidal efficacy of methanolic and n-hexane extracts of each plant species.
- To evaluate the efficacy of the botanical extracts relative to each other and to Diazinon (0.1%).

2. LITERATURAL REVIEW

2.1. Overview of Tick Infestation in Ethiopia Livestock System

Ethiopia have largest livestock production from Africa. However there are many constraints that hinder the productivity. Ectoparasites are among the one and approximately 1.49 billion livestock worldwide are susceptible to different species of Ectoparasites infestation (León *et al.*, 2020). From this ectoparasite tick are the most important which cause huge loss in the livestock production. Among this ticks family Ixodidae widely distributed and their role as vector for tick borne disease affecting livestock in tropical and subtropical country like Ethiopia (Adugna and Tamrat 2022). In Ethiopia the most predominantly distributed tick are the hard tick and in small extent soft tick. Among the genera of hard ticks affecting livestock in the country the most commonly reported include *Rhipicephalus*, *Amblyomma*, and *Hyalomma*. The recent systemic review revealed that 17,161 cattle from 41 studies reported a pooled prevalence of 64.42% (CI 57.13-71.71) for hard (ixodid) ticks and total of 82,804 adult hard ticks belonging to three different genera of ixodid ticks namely *Rhipicephalus* (47.53%), *Amblyomma* (46.10%), *Hyalomma* (6.37%) were recorded from the included studies (Girma *et al.*, 2024).

Tick affect livestock production through both direct and indirect pathways. The direct loss of tick infestation are skin damage by biting, blood loss, anemia, cause immunological reaction by inoculating of toxin, stress and discomfort on the animal (Kaba, 2022). The indirect impact are transmission of tick-borne disease, cost of treatment for clinical cases and control of tick infestation (Nath *et al.*, 2018). Additionally the consequences of tick infestation includes decrease in milk and meat production, as well as morbidity and mortality with tick-borne diseases. It can also damage skin and hides. Tick are the most important vector of pathogenic agents second after mosquitos affect human and animals. Ticks transmits diseases such as Babesiosis Theileriosis, ehrlichiosis, and borreliosis (Adane *et al.*, 2018; Rocha *et al.*, 2022)

Tick control is the important part livestock production in much of Sub-Saharan Africa. The recent large scale assessment in six African nation have confirmed medium to large resistance level to commonly used synthetic acaricides like chlorfenvinphos and amitraz, particularly from 23 stock of tick species *A. variegatum*, *R. appendiculatus*, *R. microplus* (Evans *et al.*, 2024). This resistance is due to chemical misuse by responsible resource poor farmer and pastoralist who lacks access of

veterinary oversight which leading irregular spraying interval and under- dosing. The only effective approach of tick controlling method in Ethiopia is treatment with synthetic chemical acaricides. However the widespread and misuse of accessible acaricide by the farmers and livestock owners spread acaricidal resistance (Tesfaye and Abate, 2025). The improper use of acaricides are causing widespread resistance, environmental pollution, food contamination due to inconsistent dosing and frequent misuse, and decrease the livestock production (Agegnehu, 2022). To minimize the challenges current research has focused alternative treatment tick infestation by using potential botanical acaricides like *croton macrostachyus*, and *Ricinus communis* showing high invitro acaricidal efficacy against *Rhipicephalus* and *Amblyomma* species (Fatalo and Alkadir, 2026).

2.2. Ethnoveterinary Use of Medicinal Plants in Tick Control in Ethiopia

The ethnoveterinary medicine in Ethiopia play crucial role in livestock health management, where indigenous knowledge of medicinal plants widely practiced due limited access to modern veterinary service. Over 80% of population in Africa depend on medicinal plant remedies and in Ethiopia, such practices help the treatment 90% of livestock disease including tick-borne diseases (TBDs) (Birhanu and Abera, 2015). This strong reliance is contribute accessibility, affordability, and cultural acceptability of herbal remedies among rural livestock owners and farmers. However the transmission of this knowledge from generation to generation is not enhanced, only orally which cause knowledge gaps and improper documentation. Many studies conducted in different region of Ethiopia have documented diversified plant species used to treat livestock disease (Kemal *et al.*, 2020; Beyene *et al.*, 2024; Hankiso *et al.*, 2024; Argessa *et al.*, 2025; Fatalo and Alkadir, 2026).

worldwide ticks control mainly based on repeated use of synthetic acaricides which have resulted direct and indirect problems related to development of resistance, environmental pollution, meat and milk contamination, and treatment cost (Pirali and da Silva, 2011). The use of natural products, mainly acaricide from plant source used for control tick infestation, has been focus area of research in many country (Nchu *et al.*, 2012). The scientific validation of this natural product is developed from simple botanical survey to advance in-vivo and in-vitro acaricidal activity testing. Recent pharmacological studies revealed that specific plant extract can cause significant mortality in

major tick genera like *Rhipicephalus*, and *Amblyomma* (Kemal *et al.*, 2020). The acaricidal activity of crude extract of different plant species against tick in different region of country has been reported which have explained in table below

Table 1: Summary of selected medicinal plants used against tick in Ethiopia

Plant species	Study region	Target tick species	Extraction method	Efficacy or mortality rate	citation
<i>C. aurea</i>	southern Ethiopia(Sidama region)	<i>Amblyomma variegatum</i> , <i>R. Pulchellus</i>	Ethanolic aqueous leaf extract	LC50 27 and 29 ppm, LC50 37 and 41 ppm and inhibit hatchability	(Negash <i>et al.</i> , 2024)
<i>P. schimperi</i>	Ethiopia(traditional)	Ticks general	Not specified	Traditional uses for ticks mites no quantified efficacy found	(Bekele <i>et al.</i> , 1993)
<i>R. communis</i>	Eastern Ethiopia	<i>Rhipicephalus decoloratus</i> <i>R. Pulchellus</i>	Methanolic extraction method	80% 100mg/ml	(Kemal <i>et al.</i> , 2020)
<i>A. yavellana</i>	Borana Ethiopia	<i>Amblyomma variegatum</i> larvae	Leaf latex (larval packet test)	LC50 35.82 mg/ml and LC99 83.48 mg/ml,	(Hailesillassie <i>et al.</i> , 2018)
<i>L. sativum</i>	Western Ethiopia (Keffa, Ilubabor, Wollega)	<i>R. decoloratus</i>	Crushed seed mixed with cattle feed	Traditional uses and invitro killing effect 30 – 100%	(Regassa, 2000)

P. schimperi and *L. sativum* have limited quantitative data, mainly traditional application without detailed mortality rate in peer-reviewed efficacy test.

2.3. Phytochemistry and Acaricidal Potential of Selected Plants

Calpurnia aurea: - *C. aurea* is widely used in Ethiopian ethnoveterinary medicine for treatment of livestock tick infestation and which have rich phytochemical and contents and high potential of acaricidal activity. Recent study revealed that the phytochemical screening of the plant have shown the presence of alkaloids flavonoids tannins and saponins which are contribute to its biological efficacy. Alkaloid exerts on neurotoxic effect on ticks leading to paralysis and eventually death, and flavonoids and tannin interfere with enzymatic and metabolic system of the parasite (Kemal *et al.*, 2020). According to Negash *et al.*, 2024 *C.aurea* methanolic and aqueous leaf extract showed more promising effect on tick survival hatchability than synthetic diazinon. Achieve mortality rate 80-90% against tick species such as *R. decoloratus* and *R. pulchellus*. The efficacy of this plant is influenced by concentration, and exposure time with higher doses producing significant greater mortality rate (Zorloni *et al.*, 2010).

Ricinus communis: - *R. communis* commonly used medicinal plants in Ethiopia with notable acaricidal and Ectoparasiticidal properties which contain diverse potent phytochemical composition such as ricin, alkaloids flavonoids and fatty acids which collectively contribute to its effectiveness against ticks. Ricin inhibiting protein synthesis in parasite cells leading to cellular dysfunction and death. The non-polar compounds enhance the penetration of active substances through tick cuticle. Additionally flavonoids and alkaloids interfere with metabolic and enzymatic, further toxicity (Ghosh *et al.*, 2007). Recent studies conducted in Ethiopia have demonstrated that methanolic extract of *R. communis* particularly from seed and leaves, can produce mortality rate exceeding 80% against major tick species like *R. pulchellus*, and *R.decoloratus* with efficacy increasing alongside concentration, and exposure time (Kemal *et al.*, 2020).

Premna schimperi: - Lamiaceae (*P. schimperi*) recorded in Ethiopian herbal medicine survey for ectoparasite control. The phytochemical contents of the plant rich in secondary metabolite that have anti-inflammatory, anti-oxidant, and acaricidal or repellent effects. However targeted acaricidal bioassay are limited and the combination of traditional use identifiable bioactive metabolites of *P. schimperi* claimed for invitro acaricidal testing (Isa *et al.*, 2023).

Aloe yavellana:- *Aloes* is the recognized medicinal plant in Ethiopia which have possess various biological activity including anti-microbial, anti-malarial, and anti-leishmanial (Abeje *et al.*, 2014;

Teka *et al.*, 2016; Takele, 2018). *A. yavellana* is an endemic Aloes species in Ethiopia traditionally used for treatment various illness of human and animal in Borana and southwest Ethiopia. According to Hailesillassie *et al.*, (2018), two major compound isolated from *A. yavellana* namely Aloin A/B, and Microdantin A/B were assessed for their larvicidal activity against *A. variegatum* using larval packet test (LPT) the LC50 and LC99 were found to be 35.82 ± 2.27 and 83.48 ± 3.95 mg/ml, respectively. Microdantin have higher larvicidal activity than Aloin.

Lepidium sativum :- Study in Egypt revealed that the acaricidal activity of *L. sativum* compared with *R. communis* and other seven plants, using aqueous extract of adult immersion test against *Hyalomma dromedarii* with measurable toxicity with relative efficacy 1.3 times higher the reference plant (*Lawsonia inermis*). The plants shows moderate potential botanical acaricide, supporting its possible use in tick control strategy (Abd *et al.*, 2023).

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted from November to May 2025/26 in and around Bishoftu particularly in Dirre, Chelalaka, and Yerar. The plant material was collected from Yerar Mountain east of Addis Ababa and ticks collected from cattle in Dirre, Chelalaka, and Yerar. It is located at the latitude 9°N, longitude 40°E and altitude 1850 m.a.s.l in the central highland of Ethiopia. The rainfall is bimodal. It receives annual rainfall of 886mm of which 84% received during the long rainy season covering June to September. The mean maximum and minimum temperature of the area is 26°C and 14°C respectively and mean relative humidity is 61.3% (Hunde *et al.*, 2015). Extraction preparation, and acaricidal assay was carried out in Addis Ababa University College of Veterinary Medicine and Agriculture and laboratory work extraction was performed in Aklilu Lemma Institute of Pathobiology in medicinal plant laboratory and phytochemical screening was conducted Addis Ababa university college of health science in pharmacognosy laboratory.

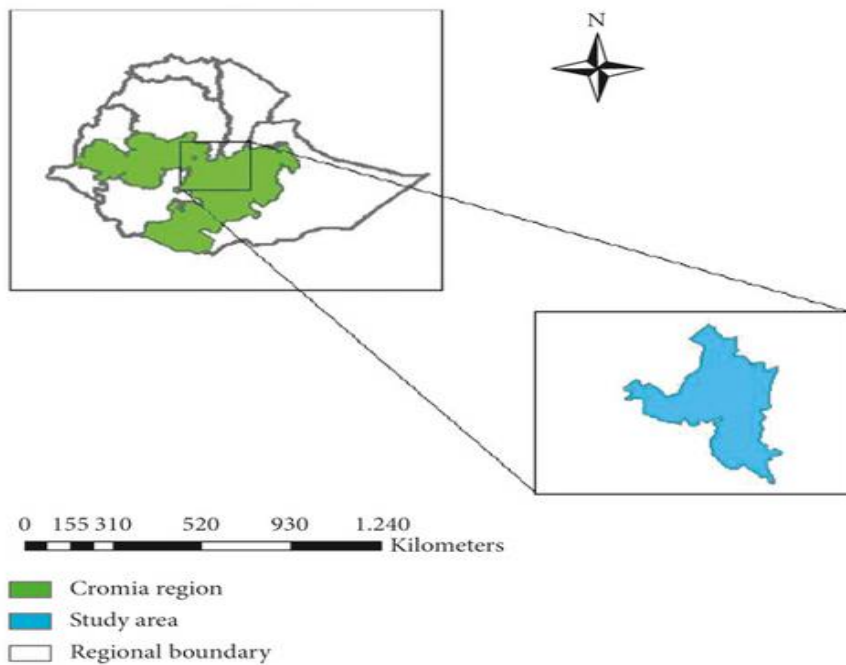


Figure 1: Map of study area

3.2. Study Design

This study was employed an experimental laboratory study design using standard invitro bioassay (adult immersion test) described by Vongkhamchanh et al., 2014 to evaluate methanol and hexane crude extract of the five selected medicinal plants.

Collection and identification of plant sample: - The plants were chosen based on scientific and ethnomedical information in the literature and additionally with a preliminary ethnobotanical survey conducted during the preparation of the research proposal. The selected plants that were included in the experiment were *C. aurea* (*digita/ceeka*), *R. communis* (*Gulo/Qobbo*), *P. schimperi* (*urgessa*), *L. sativum* (*Feeto/shimfu*), and *A. yavellana* (*Ret/Hargisa*). The selected plants were collected, identified and verified by a plant taxonomist in Addis Ababa University College of Natural and Computational Science Department of Plant Biology and Biodiversity Management (Code name: RF001, RF002, RF003, RF004). During leaf collection process latex glove were worn to minimize contamination particularly from fungi.

Crude extract preparation: - The extraction preparation, and acaricidal bioassay was conducted in Addis Ababa University College of Veterinary Medicine at pharmacology laboratory. The fresh leaf of the plants were cleaned with tap water to remove dust and dried in closed door at room temperature for two weeks, mechanically grinded and coarsely powdered by using electrical powder grinder, DE-350g, (Volt = 220V/50-60Hz, Power = 1600W, Fineness = 30-300M, RPM = 25000r/min). Then the coarse powder sieved by the 800 µm size aperture which United States Pharmacopeia (USP) standard. The extraction process was carried out in Aklilu Lemma Institute of Pathobiology medicinal plant laboratories. The coarse powdered plant specimens was then subjected to extraction using 80% methanol for polar compound, and n-hexane for non-polar compounds by cold maceration technique.

A 100g plant powder was soaked 500ml of flask with of methanol, and distilled water at ratio of 1:5 w/v. Then the mixture was shaking periodically for three days (72 hrs) with speed of 120 rpm and then filtered. The mixture was first filtered using gauze then the filtrate was passed through Whatman No. 1 filter paper. The filtrates of methanol and hexane crude extract was concentrated using vacuum rotary evaporator (Rota Vapor- RE) under reduced pressure at 40° C while the aqueous crude extract were evaporated to dryness using hot oven 40° C for two days. The pure

extract from each plant was left cooled by room temperature to remove traces solvents and finally collected separately in a Wheaton bottle glass containers and were preserved a refrigerator at 4° C until used for experimentation.

3.3. Ticks Collection and Identification

Adult tick of *R. decoloratus*, and *A. variegatum* were collected from naturally infested cattle at veterinary clinic, grazing area, livestock market, and farms of Dirre, Chelalaka, and Yerar. The entire body surface of the animal was examined thoroughly and the adult ticks were manually removed from the body of animal using fine tipped forceps and placed individually in labeled plastic 50ml ventilated tube vials carry 10 adult ticks per tube . It transported in insulated cool boxes to Addis Ababa University College of Veterinary medicine within 6 hours of collection (Holdsworth *et al.*, 2006). All collected ticks were morphologically identified at species level by using stereomicroscope. Unfed adult ticks with uniform size and were selected, cleaned by sodium hypochlorite, and maintained at relative humidity.

3.4. Acaricidal Activity Evaluation

Stock Solution Preparation: A stock solution for methanolic and n-hexane extracts of the plants are separately prepared by dissolving the extract in 1% dimethyl sulfoxides (DMSO) and 0.5% Tween 80 to facilitate the polar and non-polar compound found in the extracts, then distilled water was added to produce the desired concentration and required volume. The mixture was properly homogenized by vortexing until uniform solution was observed. Subsequently, the blank solution or serial dilution were prepared from stock solution for the different concentration (6.25, 12.5, 25, 50, and 100 mg/ml). The concentration were used to test the acaricidal efficacy of plant extract. Distilled water, Tween 80, and DMSO together as blank solution were used as negative control and 0.1% of diazinon 60% EC (Adamitullu pesticides processing, Ethiopia) used as positive control (Heukelbach *et al.*, 2006).

Adult Immersion Test: The test was started after 1 hour of tick collection. Ten adult live and active ticks in three replication were immersed in petridish, and 3ml of concentration was added to the replicated petridish for two minute of exposure. Then the tick were filtered after immersion in the separate petridish (Zaman *et al.*, 2012). The petridish were incubated under 28°C temperature, and 80% relative humidity. Each ticks were observed by stereomicroscope for any death at 30 min,

1hr, 2hr, 3hr, 6hr, 12hr, and 24hr time interval (Du *et al.*, 2008). The Viability of ticks were observed regularly by stimulation with a needle and tick were recorded as dead if no motion or reaction was shown. The mortality was calculated according to the formula given by Krishnaveni and Venkatalakshmi, 2014 as follow.

$$Mortality \% = \frac{\text{Number of dead ticks}}{\text{Total number of ticks}} * 100$$

3.5.Data Management nd Analysis

Recorded data was entered into Microsoft excel database system used for data management. R software 4.4.2 version was used for data analysis. One-way ANOVA (followed by Tukey's HSD) was used to compare mean tick mortality between different concentrations and extracts, and controls at each time point. Mortality percentages were reported as mean $\pm$ standard error. The all significance level were set as $P < 0.05$.

3.6. Phytochemical Screening Test

Phytochemical screening was carried out to identify the qualitative chemical component of crude extract of selected plants using commonly used method precipitation and coloration reaction to identify the major natural chemical groups and secondary metabolites found in the plants. Many methods combined to identify these metabolites in medicinal plant. Standard screening test were carried out in Addis Ababa University School of Pharmacy Laboratories using a standard protocol and reagents on methanol and n-hexane extract of herbs to describe the content found in them. The screening test was done to detect the major secondary metabolites or bioactive believed to have Acaricidal activities like alkaloid, saponins, flavonoids, steroid, terpenoids, and glycosides.

Dragendorff's, Mayer's, and Wagner's testes for Alkaloids: Three tests were used to identify alkaloids in plant. 2ml plant extract and 5ml of 1% HCl mixed and heated on water bath for 30 minutes then filtered. 1ml of the filtrate was taken in to 2 test tube. For the first portion few drops of Dragendorff's reagent were added Occurrence of orange red precipitate was taken as presence of alkaloids. To the second tube 1ml of filtrate and Mayer's reagent was mixed and appearance of buff-coloured precipitate was taken as positive. 2ml of plant extracts and 2ml of Wagner's reagent

was mixed and the formation of reddish color indicate presence of alkaloids in the plant extracts(Sofowora 1993).

Shinoda's tests for Flavonoids: 0.5ml of plant extracts was dissolved in ethanol warmed and then filtered. Three pieces of Mg chips was added to the filtrate, then few drops of concentrated HCl added. A pink, orange, red to purple was taken as the presence of flavonoids (Evans, 2002).

Salkowski tests for terpenoids: 2ml of plant extracts was dissolved in ethanol. 1ml of acetic anhydride added to it. Then followed by addition of conc. H₂SO₄. Color change from pink to violate was taken as the presence of terpenoids (Trease and Evans, 2002).

Liebermann-Burchard tests for steroids: 2ml of extracts, 2ml of acetic acid was added. Then add H₂SO₄ violet to blue or bluish-green was taken the presence of steroids (Sofowora 1993).

Frothing tests for Saponins: 2ml of extracts was boiled with 5ml of distilled water, filtered. To the filtrate about 3ml of distilled water were added again and mixed vigorously for 5 minutes the thickness and persistent frothy was taken as positive (Sofowora 1993)..

Test for glycosides: 1ml of plant extracts was dissolved in 2ml of distilled water, then aqueous NaOH was added. Yellow color formation indicate the presence of glycosides in the extracts (Deshmukh and Theng, 2018).

4. RESULT

4.1. Percentage of Methanol Extraction Yield

Table 2: Yield of methanolic and n- hexane extracts

Scientific name	Family	Plant part	Source	Dry weight(g)	Methanol		n-hexane	
					Yield in g	Yield in %	Yield in g	Yield in %
<i>C. aurea</i>	Fabaceae	Leaf	Bishoftu	150	14.1	9.4	2.1	1.4
<i>P. schimperi</i>	Lamiaceae	Leaf	Yerar	150	34.7	23.14	2.9	1.9
<i>R. communis</i>	Euphorbiaceae	Leaf	Bishoftu	150	18.1	12.06	3.1	2.06
<i>A. yavellana</i>	Asphodelaceae	Leaf	Bishoftu	100	3.9	3.9	1.2	1.2
<i>L. sativum</i>	Brassicaceae	Seed	Market	100	6.1	6.1	5	5

Table 2 summarize the percentage methanol extraction yield 150g for three plants *C. aurea*, *P. schimperi*, and *R. communis*, and 100g for *A. yavellana*, and *L. sativum*. The color of each extract are *C. aurea* (bright green), *P. schimperi* (dark green), *R. communis* (deep green), *A. yavellana* (dark brown), and *L. sativum* (yellowish). The highest and lowest percentages of methanolic extract yield obtained were *P. schimperi* (23.14%), and *A. yavellana* respectively. The percentage of the n-hexane extract yield with similar dry weight of methanol extract have significant difference. The highest and lowest percentage of n-hexane extract yield obtained were *L. sativum* (5%), and *A. yavellana* (1.2%).

4.2. Comparative Efficacy Between Plant Species Extracts Against Adult Ticks

The result show that tick mortality increased with increasing with concentration and exposure time which indicating a clear dose-and time dependent acaricidal effect across all plant species. From polar methanolic extract *C. aurea* exhibited the strongest acaricidal efficacy throughout the experimental period. At 100 mg/ml concentration mortality rate increased from 4.67 ± 0.33 at 30minutes to 9.00 ± 0.00 after 24 hours of exposure. The most pronounced increase occurred during the first 6 hours were mortality increase from 4.67 ± 0.33 to 8.00 ± 0.00 . The time point which were produce greatest mortality was 30 min-1hours which increase form 4.67 ± 0.33 to 6.67 ± 0.33 (+ 2 ticks)(Table 4).This suggesting strong bioactive potential of the polar constituents

extracted by methanol. The methanolic extract of *P. schimperi* showed substantial acaricidal activity however less efficacy than *C. aurea*. Mortality at 100 mg/ml increased from 3.33 ± 0.33 to 8.33 ± 0.33). The time point of greatest mortality increase were occurred at 1hrs - 2hrs, +2.33 ticks at 50mg/ml concentration, and 3-6 hours at 100mg/ml (Table 5).

L. sativam and *R. communis* moderate acaricidal activity. At high concentration (100 mg/ml) mortality increase from 2.00 ± 0.00 to 7.00 ± 0.00 at 30minutes to 24 hours for both plants (Table 6; Table 7). In contrast methanolic extract of *A. yavellana* showed the lowest acaricidal activity among the rest four plants extracted by methanol, the maximum mortality observed at 24 hours and 100 mg/ml was 6.00 ± 0.00 (Table 8). The overall methanolic extract mortality comparison of the five plant species were: - *C. aurea* > *P. schimperi* > *L. sativam*, and *R. communis* > *A. yavellana*

The n-hexane extract of *R. communis* showed comparatively higher activity, reaching 7.00 mortality rate at 100 mg/ml after 24 hrs exposure (Table 13). *P. schimperi* and *C. aurea* also demonstrate notable activity with mortality rate of 6.67 ± 0.33 and 6.33 ± 0.33 at 100 mg/ml concentration and 24 hrs exposure time (Table 9; Table 10). The lowest acaricidal efficacy among n-hexane extracts was observed in *A. yavellana*, where mortality rate at high concentration and 24 hrs exposure time reached only 5.67 ± 0.33 (Table 12). The lower acaricidal efficacy may indicate of non-polar bioactive content in this plant species. The overall n-hexane extract mortality of the five plant species were: *R. communis* > *P. schimperi* > *C. aurea* > *L. sativam* > *A. yavellana*

Overall, the present study revealed that both methanolic and n-hexane extracts of the tested plant type possess acaricidal effect against adult ticks, and the methanolic extract were greater potent or efficacy than the n-hexane extracts. The increasing of mortality rate with increasing concentration and exposure time significantly supports time- and dose dependent nature of the acaricidal effect. The methanolic extract of *C. aurea* appeared to be the highest acaricidal efficacy among the tested plant species, followed by *P. schimperi*. These present study suggest that these plants may serve as possible sources of environmentally friended acaricidal agents for tick control and further phytochemical and toxicological investigation to identify and characterize their bioactive compound.

The LC50 values indicated significant differences in acaricidal activity among the tested plant species against adult ticks, where lower LC50 values showed higher toxicity and better efficacy. Methanolic plant extracts showed more acaricidal effect than n-hexane plant extracts for all plant species, this suggesting that methanol plant extracts have more active acaricidal constituents. Among the tested plants methanolic extract of *Premna schimperi* found with highest efficacy with lowest LC50 values (2.18), followed by *R. communis* (3.84), *C. auria* (6.79), and *L. sativam* (8.58). In contrast, *A. yavellana* exhibited with lowest efficacy with the highest LC50 values in both polar and non-polar solvents (Table 14). Generally, the efficacy of the plants against adult ticks depend on LC50 was ranked as: *P. schimperi* > *R. communis* > *C. auria* > *L. sativam* > *A. yavellana*.

Table 3: In-vitro tick killing effect of methanolic extract of *C. aurea* against adult tick

Conc	Mean number of ticks dead (mean of mortality $\pm$ SE)at hour post exposure						
	30 min	1 hr	2 hr	3 hr	6 hr	12 hr	24 hr
100	4.67 $\pm$ 0.33	6.67 $\pm$ 0.33	6.67 $\pm$ 0.33	7.67 $\pm$ 0.33	8.00 $\pm$ 0.00	8.67 $\pm$ 0.33	9.00 $\pm$ 0.00
50	2.67 $\pm$ 0.33	5.33 $\pm$ 0.33	6.00 $\pm$ 0.00	6.67 $\pm$ 0.33	7.33 $\pm$ 0.33	7.33 $\pm$ 0.33	7.67 $\pm$ 0.33
25	0.67 $\pm$ 0.33	2.00 $\pm$ 0.58	4.67 $\pm$ 0.33	5.00 $\pm$ 0.00	5.33 $\pm$ 0.33	6.00 $\pm$ 0.00	6.00 $\pm$ 0.00
12.5	0.67 $\pm$ 0.33	1.00 $\pm$ 0.00	3.33 $\pm$ 0.33	4.00 $\pm$ 0.58	4.33 $\pm$ 0.33	5.00 $\pm$ 0.58	6.00 $\pm$ 0.00
6.25	0.00 $\pm$ 0.00	0.67 $\pm$ 0.33	1.00 $\pm$ 0.00	1.67 $\pm$ 0.33	4.33 $\pm$ 0.33	4.33 $\pm$ 0.33	5.33 $\pm$ 0.33
Diaz(0.1)	0.60 $\pm$ 0.33	1.80 $\pm$ 0.33	3.00 $\pm$ 0.00	4.13 $\pm$ 0.33	5.33 $\pm$ 0.33	6.73 $\pm$ 0.33	8.00 $\pm$ 0.00
Distilled water	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.13 $\pm$ 0.33

Table 4: In-vitro tick killing effect of methanolic extract of *P. schimperi* against adult tick

Mean number of ticks dead (mean of mortality $\pm$ SE)at hour post exposure							
Conc	30 min	1 hr	2 hr	3 hr	6 hr	12 hr	24 hr
100	3.33 $\pm$ 0.33	5.33 $\pm$ 0.33	5.33 $\pm$ 0.33	6.33 $\pm$ 0.33	6.67 $\pm$ 0.33	7.33 $\pm$ 0.33	8.33 $\pm$ 0.33
50	2.00 $\pm$ 0.00	2.67 $\pm$ 0.33	5.00 $\pm$ 0.00	5.33 $\pm$ 0.33	6.00 $\pm$ 0.00	6.33 $\pm$ 0.33	7.33 $\pm$ 0.33
25	1.67 $\pm$ 0.33	2.33 $\pm$ 0.33	3.00 $\pm$ 0.00	5.00 $\pm$ 0.00	5.67 $\pm$ 0.33	6.33 $\pm$ 0.33	7.00 $\pm$ 0.00
12.5	1.00 $\pm$ 0.00	1.67 $\pm$ 0.33	3.00 $\pm$ 0.00	3.33 $\pm$ 0.33	5.33 $\pm$ 0.33	5.67 $\pm$ 0.33	6.67 $\pm$ 0.33
6.25	0.67 $\pm$ 0.33	1.33 $\pm$ 0.33	2.33 $\pm$ 0.33	2.67 $\pm$ 0.33	3.67 $\pm$ 0.33	4.00 $\pm$ 0.00	6.00 $\pm$ 0.00
Diaz(0.1)	0.60 $\pm$ 0.33	1.80 $\pm$ 0.33	3.00 $\pm$ 0.00	4.13 $\pm$ 0.33	5.33 $\pm$ 0.33	6.73 $\pm$ 0.33	8.00 $\pm$ 0.00
Distilled water	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.13 $\pm$ 0.33

Table 5: In-vitro tick killing effect of methanolic extract of *L. sativam* against adult tick

Mean number of ticks dead (mean of mortality $\pm$ SE)at hour post exposure							
Conc	30 min	1 hr	2 hr	3 hr	6 hr	12 hr	24 hr
100	2.00 $\pm$ 0.00	2.33 $\pm$ 0.33	5.00 $\pm$ 0.00	5.33 $\pm$ 0.33	6.33 $\pm$ 0.33	6.67 $\pm$ 0.33	7.00 $\pm$ 0.00
50	1.00 $\pm$ 0.00	2.00 $\pm$ 0.00	2.67 $\pm$ 0.33	3.33 $\pm$ 0.33	6.00 $\pm$ 0.00	6.33 $\pm$ 0.33	6.67 $\pm$ 0.33
25	0.67 $\pm$ 0.33	1.00 $\pm$ 0.00	2.33 $\pm$ 0.33	3.00 $\pm$ 0.00	3.33 $\pm$ 0.33	5.33 $\pm$ 0.33	6.00 $\pm$ 0.00
12.5	0.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.67 $\pm$ 0.33	2.33 $\pm$ 0.33	3.00 $\pm$ 0.00	5.00 $\pm$ 0.00	5.33 $\pm$ 0.33
6.25	0.00 $\pm$ 0.00	0.33 $\pm$ 0.33	0.67 $\pm$ 0.33	1.33 $\pm$ 0.33	2.00 $\pm$ 0.00	2.67 $\pm$ 0.33	4.67 $\pm$ 0.33
Diaz(0.1)	0.60 $\pm$ 0.33	1.80 $\pm$ 0.33	3.00 $\pm$ 0.00	4.13 $\pm$ 0.33	5.33 $\pm$ 0.33	6.73 $\pm$ 0.33	8.00 $\pm$ 0.00
Distilled water	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.13 $\pm$ 0.33

Table 6: In-vitro tick killing effect of methanolic extract of *R. communis* against adult tick

Mean number of ticks dead (mean of mortality $\pm$ SE)at hour post exposure							
Conc	30 min	1 hr	2 hr	3 hr	6 hr	12 hr	24 hr
100	2.00 $\pm$ 0.00	2.33 $\pm$ 0.33	5.00 $\pm$ 0.00	5.33 $\pm$ 0.33	6.33 $\pm$ 0.33	6.67 $\pm$ 0.33	7.00 $\pm$ 0.00
50	1.00 $\pm$ 0.00	2.00 $\pm$ 0.00	2.67 $\pm$ 0.33	3.33 $\pm$ 0.33	6.00 $\pm$ 0.00	6.33 $\pm$ 0.33	6.67 $\pm$ 0.33
25	0.67 $\pm$ 0.33	1.00 $\pm$ 0.00	2.33 $\pm$ 0.33	3.00 $\pm$ 0.00	3.33 $\pm$ 0.33	5.33 $\pm$ 0.33	6.00 $\pm$ 0.00
12.5	0.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.67 $\pm$ 0.33	2.33 $\pm$ 0.33	3.00 $\pm$ 0.00	5.00 $\pm$ 0.00	5.33 $\pm$ 0.33
6.25	0.00 $\pm$ 0.00	0.33 $\pm$ 0.33	0.67 $\pm$ 0.33	1.33 $\pm$ 0.33	2.00 $\pm$ 0.00	2.67 $\pm$ 0.33	4.67 $\pm$ 0.33
Diaz(0.1)	0.60 $\pm$ 0.33	1.80 $\pm$ 0.33	3.00 $\pm$ 0.00	4.13 $\pm$ 0.33	5.33 $\pm$ 0.33	6.73 $\pm$ 0.33	8.00 $\pm$ 0.00
Distilled water	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.13 $\pm$ 0.33

Table 7: In-vitro tick killing effect of methanolic extract of *A. yavellana* against adult tick

Mean number of ticks dead (mean of mortality $\pm$ SE)at hour post exposure							
Conc	30 min	1 hr	2 hr	3 hr	6 hr	12 hr	24 hr
100	1.33 $\pm$ 0.33	4.00 $\pm$ 0.00	4.33 $\pm$ 0.33	4.67 $\pm$ 0.33	5.00 $\pm$ 0.00	5.67 $\pm$ 0.33	6.00 $\pm$ 0.00
50	1.00 $\pm$ 0.00	3.00 $\pm$ 0.00	3.33 $\pm$ 0.33	3.67 $\pm$ 0.33	4.00 $\pm$ 0.00	4.67 $\pm$ 0.33	5.67 $\pm$ 0.33
25	0.67 $\pm$ 0.33	1.67 $\pm$ 0.33	2.00 $\pm$ 0.00	3.00 $\pm$ 0.00	3.33 $\pm$ 0.33	4.00 $\pm$ 0.00	5.00 $\pm$ 0.00
12.5	0.00 $\pm$ 0.00	1.00 $\pm$ 0.00	2.00 $\pm$ 0.00	2.00 $\pm$ 0.00	2.33 $\pm$ 0.33	4.33 $\pm$ 0.33	4.67 $\pm$ 0.33
6.25	0.00 $\pm$ 0.00	0.33 $\pm$ 0.33	2.00 $\pm$ 0.00	2.33 $\pm$ 0.33	3.00 $\pm$ 0.00	3.00 $\pm$ 0.00	4.33 $\pm$ 0.33
Diaz(0.1)	0.60 $\pm$ 0.33	1.80 $\pm$ 0.33	3.00 $\pm$ 0.00	4.13 $\pm$ 0.33	5.33 $\pm$ 0.33	6.73 $\pm$ 0.33	8.00 $\pm$ 0.00
Distilled water	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.13 $\pm$ 0.33

Table 8: In-vitro tick killing effect of n-Hexane extract of *C. aurea* against adult tick

Mean number of ticks dead (mean of mortality $\pm$ SE)at hour post exposure							
Conc	30 min	1 hr	2 hr	3 hr	6 hr	12 hr	24 hr
100	1.67 $\pm$ 0.33	3.67 $\pm$ 0.33	4.33 $\pm$ 0.33	5.00 $\pm$ 0.00	5.67 $\pm$ 0.33	6.33 $\pm$ 0.33	6.33 $\pm$ 0.33
50	1.00 $\pm$ 0.00	1.67 $\pm$ 0.33	2.67 $\pm$ 0.33	4.67 $\pm$ 0.33	4.67 $\pm$ 0.33	5.67 $\pm$ 0.33	6.00 $\pm$ 0.00
25	0.00 $\pm$ 0.00	1.00 $\pm$ 0.00	2.33 $\pm$ 0.33	2.67 $\pm$ 0.33	4.67 $\pm$ 0.33	4.67 $\pm$ 0.33	5.33 $\pm$ 0.33
12.5	0.00 $\pm$ 0.00	0.33 $\pm$ 0.33	1.33 $\pm$ 0.33	2.33 $\pm$ 0.33	2.33 $\pm$ 0.33	4.00 $\pm$ 0.00	5.00 $\pm$ 0.00
6.25	0.00 $\pm$ 0.00	0.33 $\pm$ 0.33	0.33 $\pm$ 0.33	1.33 $\pm$ 0.33	2.33 $\pm$ 0.33	3.33 $\pm$ 0.33	3.67 $\pm$ 0.33
Diaz(0.1)	0.60 $\pm$ 0.33	1.80 $\pm$ 0.33	3.00 $\pm$ 0.00	4.13 $\pm$ 0.33	5.33 $\pm$ 0.33	6.73 $\pm$ 0.33	8.00 $\pm$ 0.00
Distilled water	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.13 $\pm$ 0.33

Table 9: In-vitro tick killing effect of n-Hexane extract of *P. schimperi* against adult tick

Mean number of ticks dead (mean of mortality $\pm$ SE)at hour post exposure							
Conc	30 min	1 hr	2 hr	3 hr	6 hr	12 hr	24 hr
100	2.00 $\pm$ 0.00	4.33 $\pm$ 0.33	4.33 $\pm$ 0.33	5.33 $\pm$ 0.33	6.00 $\pm$ 0.00	6.67 $\pm$ 0.33	6.67 $\pm$ 0.33
50	1.67 $\pm$ 0.33	2.00 $\pm$ 0.00	4.00 $\pm$ 0.00	4.33 $\pm$ 0.33	5.00 $\pm$ 0.00	5.33 $\pm$ 0.33	6.33 $\pm$ 0.33
25	1.33 $\pm$ 0.33	1.67 $\pm$ 0.33	2.67 $\pm$ 0.33	3.33 $\pm$ 0.33	5.33 $\pm$ 0.33	5.67 $\pm$ 0.33	6.00 $\pm$ 0.00
12.5	1.00 $\pm$ 0.00	1.67 $\pm$ 0.33	2.67 $\pm$ 0.33	3.00 $\pm$ 0.00	3.67 $\pm$ 0.33	5.67 $\pm$ 0.33	5.67 $\pm$ 0.33
6.25	0.33 $\pm$ 0.33	0.67 $\pm$ 0.33	1.67 $\pm$ 0.33	2.00 $\pm$ 0.00	2.67 $\pm$ 0.33	3.67 $\pm$ 0.33	5.00 $\pm$ 0.00
Diaz(0.1)	0.60 $\pm$ 0.33	1.80 $\pm$ 0.33	3.00 $\pm$ 0.00	4.13 $\pm$ 0.33	5.33 $\pm$ 0.33	6.73 $\pm$ 0.33	8.00 $\pm$ 0.00
Distilled water	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.13 $\pm$ 0.33

Table 10: In-vitro tick killing effect of n-Hexane extract of *L. sativam* against adult tick

Mean number of ticks dead (mean of mortality $\pm$ SE)at hour post exposure							
Conc	30 min	1 hr	2 hr	3 hr	6 hr	12 hr	24 hr
100	1.33 $\pm$ 0.33	2.00 $\pm$ 0.00	2.33 $\pm$ 0.33	4.67 $\pm$ 0.33	5.33 $\pm$ 0.33	6.00 $\pm$ 0.00	6.33 $\pm$ 0.33
50	0.67 $\pm$ 0.33	1.67 $\pm$ 0.33	2.33 $\pm$ 0.33	2.67 $\pm$ 0.33	4.33 $\pm$ 0.33	5.33 $\pm$ 0.33	5.67 $\pm$ 0.33
25	0.33 $\pm$ 0.33	0.67 $\pm$ 0.33	1.67 $\pm$ 0.33	2.33 $\pm$ 0.33	4.00 $\pm$ 0.00	5.00 $\pm$ 0.00	5.33 $\pm$ 0.33
12.5	0.00 $\pm$ 0.00	0.67 $\pm$ 0.33	1.33 $\pm$ 0.33	1.67 $\pm$ 0.33	2.33 $\pm$ 0.33	4.33 $\pm$ 0.33	4.67 $\pm$ 0.33
6.25	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.33 $\pm$ 0.33	1.00 $\pm$ 0.00	2.00 $\pm$ 0.00	2.33 $\pm$ 0.33	4.33 $\pm$ 0.33
Diaz(0.1))	0.60 $\pm$ 0.33	1.80 $\pm$ 0.33	3.00 $\pm$ 0.00	4.13 $\pm$ 0.33	5.33 $\pm$ 0.33	6.73 $\pm$ 0.33	8.00 $\pm$ 0.00
Distilled water	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.13 $\pm$ 0.33

Table 11: In-vitro tick killing effect of n-Hexane extract of *A. yavellana* against adult tick

Mean number of ticks dead (mean of mortality $\pm$ SE)at hour post exposure							
Conc	30 min	1 hr	2 hr	3 hr	6 hr	12 hr	24 hr
100	1.00 $\pm$ 0.00	3.33 $\pm$ 0.33	4.00 $\pm$ 0.00	4.33 $\pm$ 0.33	4.67 $\pm$ 0.33	5.00 $\pm$ 0.00	5.67 $\pm$ 0.33
50	0.67 $\pm$ 0.33	1.67 $\pm$ 0.33	2.67 $\pm$ 0.33	3.33 $\pm$ 0.33	3.67 $\pm$ 0.33	4.33 $\pm$ 0.33	5.00 $\pm$ 0.00
25	0.33 $\pm$ 0.33	1.33 $\pm$ 0.33	1.67 $\pm$ 0.33	2.67 $\pm$ 0.33	3.00 $\pm$ 0.00	3.67 $\pm$ 0.33	4.67 $\pm$ 0.33
12.5	0.00 $\pm$ 0.00	0.67 $\pm$ 0.33	1.67 $\pm$ 0.33	2.00 $\pm$ 0.00	2.00 $\pm$ 0.00	4.00 $\pm$ 0.00	4.00 $\pm$ 0.00
6.25	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	1.33 $\pm$ 0.33	2.00 $\pm$ 0.00	2.33 $\pm$ 0.33	2.67 $\pm$ 0.33	3.33 $\pm$ 0.33
Diaz(0.1))	0.60 $\pm$ 0.33	1.80 $\pm$ 0.33	3.00 $\pm$ 0.00	4.13 $\pm$ 0.33	5.33 $\pm$ 0.33	6.73 $\pm$ 0.33	8.00 $\pm$ 0.00
Distilled water	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.13 $\pm$ 0.33

Table 12: In-vitro tick killing effect of n-Hexane extract of *R. communis* against adult tick

Conc	Mean number of ticks dead (mean of mortality $\pm$ SE)at hour post exposure						
	30 min	1 hr	2 hr	3 hr	6 hr	12 hr	24 hr
100	1.67 $\pm$ 0.33	3.67 $\pm$ 0.33	4.00 $\pm$ 0.00	5.33 $\pm$ 0.33	6.33 $\pm$ 0.33	6.33 $\pm$ 0.33	7.00 $\pm$ 0.00
50	1.33 $\pm$ 0.33	1.67 $\pm$ 0.33	2.67 $\pm$ 0.33	4.67 $\pm$ 0.33	5.00 $\pm$ 0.00	5.67 $\pm$ 0.33	6.33 $\pm$ 0.33
25	0.33 $\pm$ 0.33	1.00 $\pm$ 0.00	1.33 $\pm$ 0.33	2.33 $\pm$ 0.33	4.33 $\pm$ 0.33	4.67 $\pm$ 0.33	6.00 $\pm$ 0.00
12.5	0.00 $\pm$ 0.00	0.33 $\pm$ 0.33	1.00 $\pm$ 0.00	1.33 $\pm$ 0.33	2.33 $\pm$ 0.33	4.33 $\pm$ 0.33	5.67 $\pm$ 0.33
6.25	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.67 $\pm$ 0.33	1.00 $\pm$ 0.00	2.00 $\pm$ 0.00	2.33 $\pm$ 0.33	4.67 $\pm$ 0.33
Diaz(0.1)	0.60 $\pm$ 0.33	1.80 $\pm$ 0.33	3.00 $\pm$ 0.00	4.13 $\pm$ 0.33	5.33 $\pm$ 0.33	6.73 $\pm$ 0.33	8.00 $\pm$ 0.00
Distilled water	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.13 $\pm$ 0.33

Table 13: LC₅₀ values of the plant extracts

Plant	Solvent	LC ₅₀	Confident interval
<i>C. aurea</i>	Methanol	6.79	
	n-Hexane	18.64	
<i>P. schimperi</i>	Methanol	2.18	
	n-Hexane	5.07	
<i>R. communis</i>	Methanol	3.84	
	n-Hexane	7.53	
<i>A. yavellana</i>	Methanol	20.17	
	n-Hexane	44.35	
<i>L. sativam</i>	Methanol	8.58	
	n-Hexane	17.24	

4.3. Comparative Efficacy of Plant Extracts and Diazinon Against Adult Tick

The comparative analysis showed that the acaricidal activity of most plant extracts were significantly differed from Diazinon. The methanolic extract of *C. aurea* at 100mg/ml exceeding diazinon from 30 minutes onwards. At 24 hours, the 100mg/ml concentration produced higher

mortality (9.00 ± 0.00) than diazinon (8.00 ± 0.00) indicating higher efficacy. *P. schimperi* extract mortality exceeded diazinon during the early period 30 min – 3 hours and remain comparable thereafter. At 24 hours, mortality (8.33 ± 0.33) was slightly higher than diazinon (8.00 ± 0.00). *A. yavellana* ($P < 0.001$), *L. sativam* ($P < 0.001$), and *R. communis* ($P < 0.001$) exhibited significant lower tick mortality compared with Diazinon (Figure 4). *C. aurea* ($P = 0.2752$) and *P. schimperi* ($P = 0.8530$) showed no significant difference from diazinon, indicating comparable but slightly higher acaricidal efficacy than diazinon.

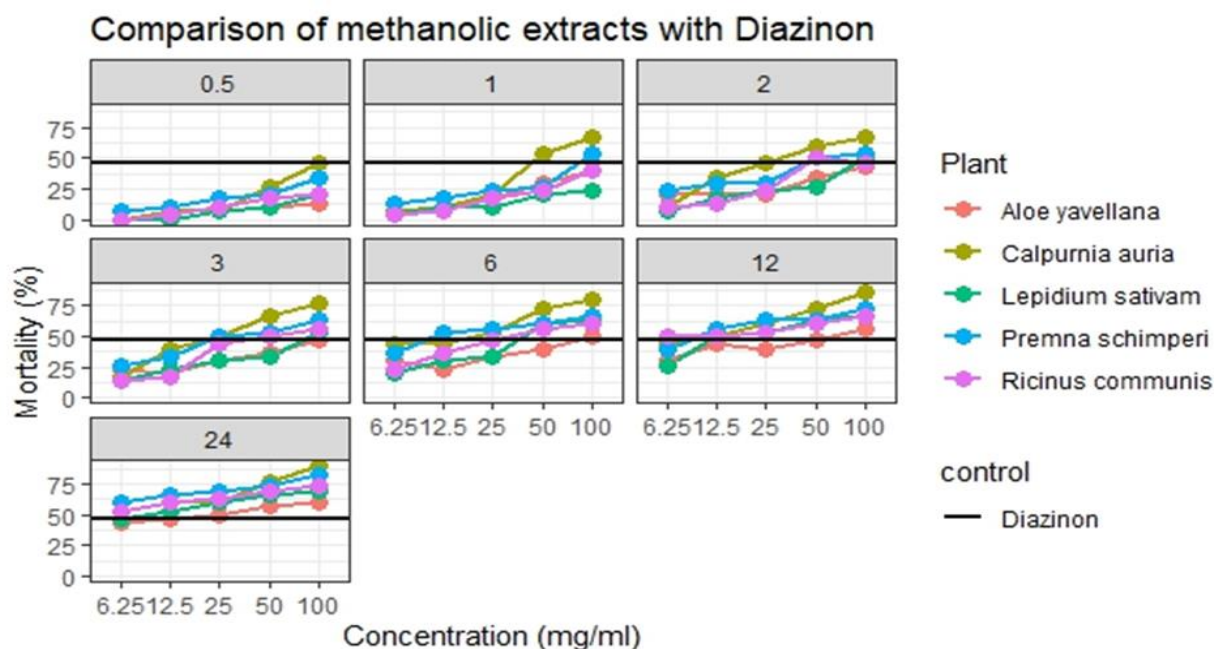


Figure 2: Dose dependent Acaricidal activity methanol extracts

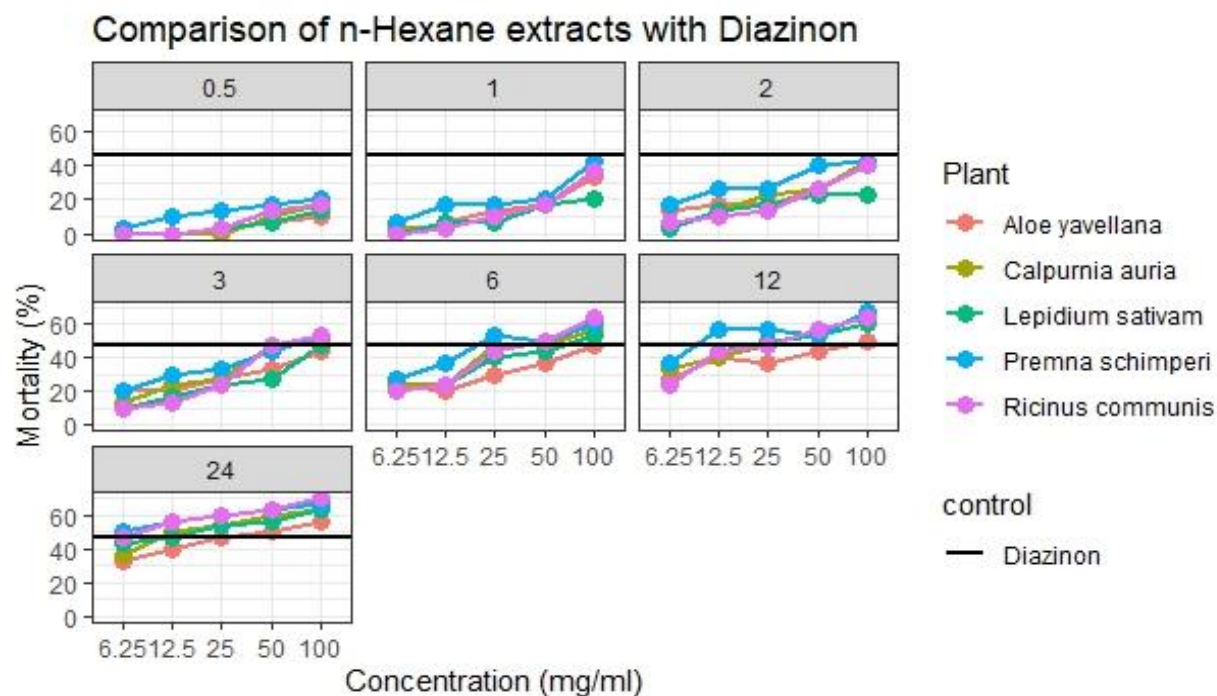


Figure 3: Dose dependent Acaricidal activity n-hexane extracts

Time-dependent of methanolic extract at 100 mg/ml

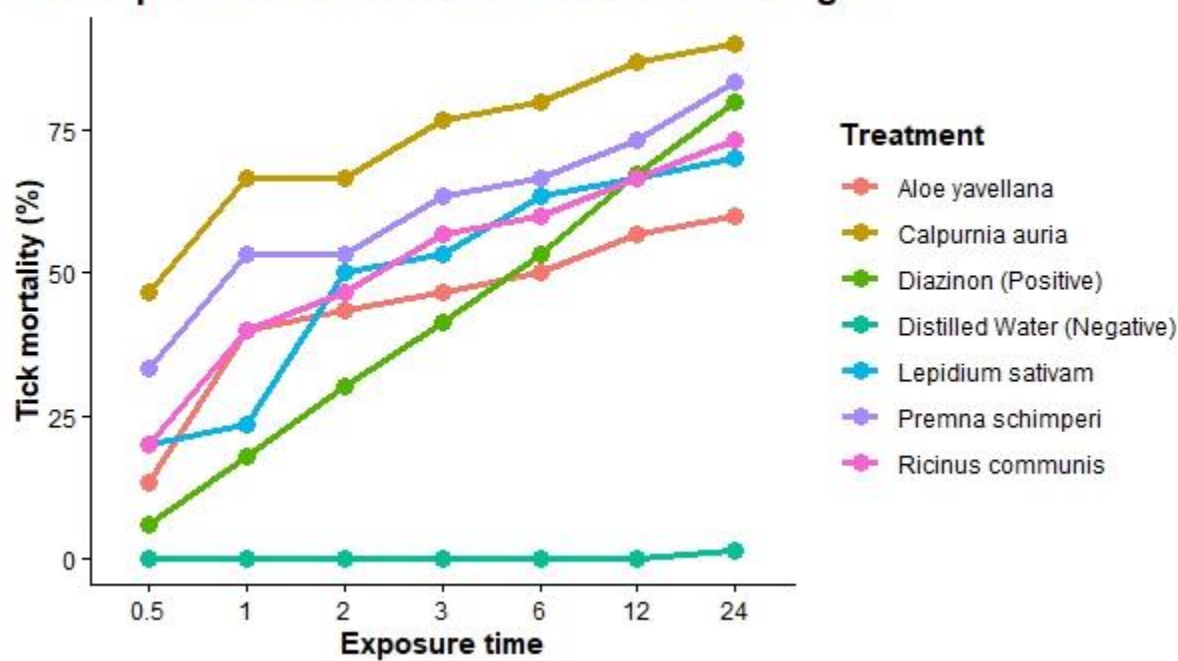


Figure 4: Time dependent methanolic extracts activity at 100mg/ml

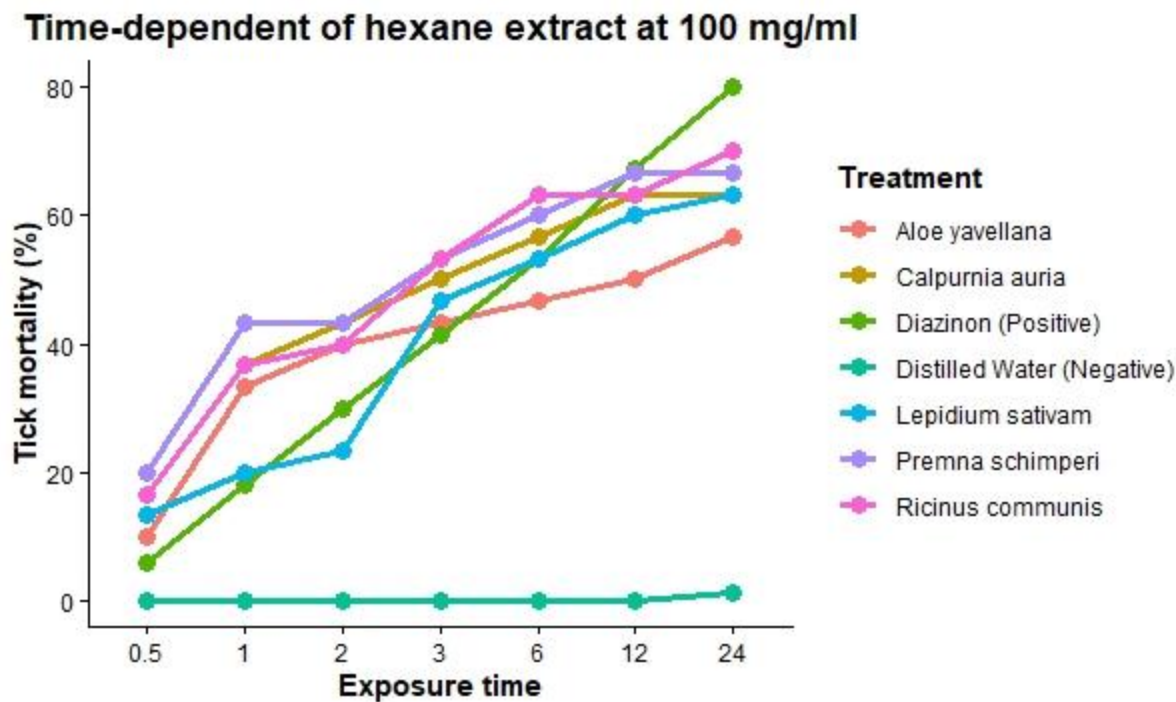


Figure 5: Time dependent n-Hexane extracts at 100mg/ml

4.4. Phytochemical Screening Test

The present study revealed that the qualitative phytochemical screening test of the methanolic extracts of the five plants showed six positive major secondary metabolites alkaloids, flavonoids, terpenoids, saponins, glycosides, and steroids. However in the n-hexane extracts there was limited secondary metabolites which was steroids and terpenoids whereas in the methanolic extracts of *C. aurea* positive with alkaloids (Table 3). The test type used to detect the presence of alkaloids (Mayer, Wagner, and Dragendorff's tests), Flavonoids (Shinoda test) Terpenoids (Salkowski test), Saponins (Frothing test), Glycosides (glycosides test), and Steroids (Liebermann-Burchard).

Table 14: Qualitative phytochemical Screening test

Phytochemical class	<i>C. aurea</i>		<i>P. schimperi</i>		<i>R. communis</i>		<i>L. sativam</i>		<i>A. yavellana</i>	
	ME	HE	ME	HE	ME	HE	ME	HE	ME	HE
Alkaloids	+	+	+	-	+	-	+	-	+	-
Flavonoids	+	-	+	-	+	-	+	-	+	-
Terpenoids	+	+	+	+	+	+	+	+	+	+
Saponins	+	-	+	-	+	-	+	-	+	-
Glycoside	+	-	+	-	+	-	+	-	+	-
Steroids	+	+	+	+	+	+	+	+	+	+

ME = methanol extract, HE = n-hexane extract, + = present, - = absent

5. DISCUSSION

This study revealed that there was a difference yield percentage among plant species of the same solvent, and between methanolic and n-hexane extracts. The percentage yield of the plant was higher in methanolic extraction method, this may attributed to its polar nature which enable it to dissolve broader range of phytochemical constituents including alkaloids, flavonoids, glycosides, and saponins. These were reported as major secondary metabolites in medicinal plant, and are responsible for biological effect. conversely n-hexane is a non-polar solvent that primary extract lipophilic compounds such as waxes, fats, oils, terpenoids, and certain steroids resulting in comparatively lower extraction yields. This finding agree with numerous phytochemical studies reporting higher extract recovery using polar solvent than non-polar solvents. For example Kumar *et al.*, (2011) reported that extraction yield is strongly influenced by solvent polarity and that polar solvents generally provides superior recovery of bioactive compounds from plant materials.

The present study revealed that in methanolic extracts of *C. aurea*, and *R. communis* percentage yield were 9.40%, and 12.06% respectively, this in contrast with Kemal *et al.*, (2020) which was reported 20.67%, and 30.67% respectively. The methanolic of and n-hexane extracts of *L. sativum* was 6.1%, and 5% this yield is lower were lower than those reported by Chatoui *et al.*, (2020) who recorded methanolic and n-hexane extracts 25%, and 19% respectively. This difference due to many factor, including geographical origin, environmental and climatic condition, plant age, harvesting season, solvent to sample ratio, drying condition, extraction technique, and extraction duration.

The present study showed that all methanolic extracts contained alkaloids, flavonoids, steroids, saponins, glycosides, and terpenoids. This finding are consistent with past study of phytochemical investigation of medicinal plants which have shown that methanol extracts contain wide range of polar and semi polar compounds. according to Kemal *et al.*, (2020) *C. aurea* contain flavonoids, saponins, glycosides, steroids, and alkaloids which is similar with present finding. In contrast in the same studies *R. communis* have no steroids, and alkaloids. The difference might be due to environmental and geographical variations and testing protocol. However the same result with Fatalo and Alkadir, (2026). The previous studies on phytochemical content *A. yavellana* (Addisu *et al.*, 2016) show that alkaloids, saponins, steroids, and flavonoids. according to (K Chatoui *et al.*,

2016; Kumar *et al.*, 2020) *L. sativum* have the six major secondary metabolites studied in present finding. The current finding also similar with the reports of Arega *et al.*, (2023) on phytochemical content *P. schimperi*

The present finding revealed that n-hexane extracts contained terpenoids and steroids. The n-hexane extracts of *C. aurea* contained alkaloids, steroids, and terpenoids. similarly (Tebabal and Kebede, 2021) reports the hexane extracts of *C. aurea* contained terpenoids, flavonoids, and alkaloids. *L. sativum* contain terpenoids, and steroids in contrast Kumar *et al.*, 2020) reports presence of glycosides. The difference is might be due to test protocol and environmental variation.

The current study revealed that the mean mortality of adult ticks was increased with concentration, and exposure time. This result is similar with the reports of Kemal *et al.*, (2020), Qwarse, (2015) in which tick mortality effect of plant extracts was indicated to be dose- and time dependent. Our study also showed that all the methanolic and n-hexane extracts of the tested plants at tested concentration induce significant acaricidal activities against adult ticks. The invitro tick killing activity of methanolic, and n-hexane extracts of *Calpurnia aurea* was increased with concentration, and exposure time. After 24 hours post exposure, and high concentration (100mg/ml) the methanolic extracts of the *C. aurea* showed high mortality (9.00 ± 0.00) which was slightly higher than 0.1% diazinon positive control (8.00 ± 0.00). the present result is comparable with Kemal *et al.*, (2020) which reported that mortality (8.33 ± 0.33), and Fouche *et al.*, (2017) reported that the acetone and ethanol extract of *C. aurea* had mortality effect 92.2%, and 81.1% respectively against adult ticks. Difference among studies might be due to different in solvents.

The invitro killing effect *R. communis* against adult ticks at 24 hours and high concentration (100mg/ml) was 7.00 ± 0.00 which in line with study with Kemal *et al.*, (2020) reported that The methanolic extract of *R. communis* was 7.33 ± 0.33 , however slight difference with the study of Fatalo and Alkadir, (2026) reports the mean mortality of *R. communis* against *Amblyomma* ($80.00 \pm 1.00\%$), and against *Rhipicephalus* ($73.3 \pm 0.58\%$). the difference might be due to the targeted tick species, and the parts of the plant used for extraction. The methanolic seed extract of *L. sativum* was showed 7.00 ± 0.00 mortality (Table 6). Which was comparable with positive control diazinon (8.00 ± 0.00). The present finding is consistent with a study conducted on *L. sativum* seed extracts against arthropods pests, where exhibited significant toxicity that increase with concentration, and

exposure time indicating dose-dependent bioactivity of the plant's compound content. However, the acaricidal activity observed in the present study was lower than the activity reported in some studies da Silva *et al.*, (2022) using high concentration, and at 24 hours exposure time the allium sativum had complete tick mortality 100%, this difference due to the plant species difference.

The present finding revealed that the methanolic and n-hexane extracts of *P. schimperi* produce mortality 8.33 ± 0.33 , and 6.67 ± 0.33 (Table 5, Table 10) respectively. The methanolic extract is better at killing ticks than the n-Hexane extract. This suggests that the main ingredients in *P. schimperi* that kill ticks are compounds that mix well with methanol. This is even better than the positive control diazinon which killed 8.00 ± 0.00 adult ticks. People in Ethiopia in the Sidama region have been using *P. schimperi* leaves to control ticks for a long time. They crush the leaves. Apply them to the skin. Our experimental results support this use of *P. schimperi* for managing ticks.

The invitro tick killing evaluation of *A. yavellana* leaf extract reveals a clear dose- and time-dependent acaricidal effect. The methanolic extract showed stronger potency than n-hexane extracts according to Table 8 the methanolic extract reach its maximum efficacy at the 100mg/ml dose and 24 hours post exposure produce a mean tick mortality of 6.00 ± 0.00 , whereas at the similar time and standard dose 0.1% of the positive control diazinon caused higher mortality of 8.00 ± 0.00 . In comparison (Table 12) shows that the non-polar n-hexane extract was lower efficient producing maximum mean mortality of 5.67 ± 0.33 at high concentration, and 24 hours post-exposure. This finding are strongly supported by published literature in Ethiopia flora, for instance study by Hailesillassie et al., (2018) highlighted that *A. yavellana* products are highly effective against *Amblyomma variegatum* tick and related research in south Africa reported that the *A. rupestris* has similarly confirmed a measurable tick mortality 66.6% (Adenubi et al. 2019).

The LC_{50} values obtained in the present study showed that methanolic were generally more potent than n-hexane extracts against adult tick. Among the all tested plants *Premna schimperi* produce lowest LC_{50} value (2.18mg/ml) in methanolic extract, followed by *Ricinus Communis* (3.84mg/ml) indicating strong acaricidal effect. In contrast *A. yavellana* showed the highest LC_{50} value in both methanol (20.17mg/ml) and n-hexane (44.35mg/ml) extracts. This suggesting comparatively lower efficacy. The current finding are different with previous studies (Adenubi et al. 2018) reporting

strong acaricidal activity of *Calpurnia aurea* extract against ticks with isolated Isorhoifolin ($LC_{50} = 0.65\text{mg/ml}$). Studies from Ethiopia demonstrated significant acaricidal effects of *C. aurea* and *R. communis* (Fatalo and Alkadir, (2026) particularly in polar solvent extracts supporting the lower LC_{50} values observed in the present work.

According to Hailesillassie et al., (2018) the LC_{50} of *A. yavellana* with isolated compound Aloin, and Microdantin was 35.82 ± 2.27 , and 83.48 ± 3.95 mg/ml respectively which was higher than the current finding (20.17mg/ml) and (44.35mg/ml) this difference is due to stage of the ticks used which is larvae and isolated compounds.

There is limited studies on the acaricidal activity of *P. schimperi*. However, Habtemariam, (2003) studies on antileishmanial effect of *P. schimperi* reported $LC_{50} = 1.08\mu\text{g/mL}$ with high potent diterpens compound which was comparable with the current study with $LC_{50} = 2.18\text{mg/ml}$. according to present finding the LC_{50} of methanolic and n-hexane extract of *L. sativum* was 8.58mg/ml, and 17.24mg/ml respectively. There is scarce studies done on acaricidal activity of this plants but Razavi *et al.*, (2015) reported on efficacy of *L. latifolium* against *Verroa destructor* the acaricidal activity was 100% after 12 days. However it is difficult to compare with current finding because the difference in exposure time, type of parasite, type of plant species. This give us some hint that the *Lepidium* plant have strong acaricidal effect which need more investigation.

6. CONCLUSION AND RECOMMENDATION

The current study revealed that all tested medicinal plants possessed acaricidal activity against adult ticks with efficacy increasing with concentration and exposure time increased. Methanolic extracts consistently produced greater acaricidal activity than n-hexane extracts, suggesting that polar bioactive compounds play major role in tick mortality. Among the tested plants *P. schimperi* exhibited the strongest acaricidal potency with the lowest LC50 value (2.18mg/mL). At higher concentration 100mg/mL and 24 hours post exposure methanolic extracts of *C. aurea* and *P. schimperi* produced mortality rate comparable to or higher than positive control diazinon 0.1% . This finding scientifically validate the traditional use of tested plants for tick control and indicate their potential as alternative botanical acaricides for sustainable livestock production.

Based on this finding the following recommendation should forwarded:-

- Isolate and characterize the bioactive compounds responsible for acaricidal activity, particularly *P. schimperi*, and *C. aurea*.
- Assess the safety and toxicity of the extracts and their active compounds for use in livestock.
- Evaluate efficacy against different tick life stages (larvae and nymphs) and under field conditions, including formulation and dosage optimization studies.

7. REFERENCES

- (CSA), Central Statistical Agency. (2017): “Agricultural Sample Survey.” *Volume II: Report on Livestock and Livestock Characteristics (Private Peasant Holdings)*, Statistical Bulletin 570. Ethiopia.
- Abbas, Rao Z, Muhammad Arfan Zaman, Douglas D Colwell, John Gilleard, and Zafar Iqbal. (2014): “Acaricide Resistance in Cattle Ticks and Approaches to Its Management: The State of Play.” *Veterinary Parasitology* **203** (1–2): 6–20.
- Abd Elgawad, Samah, Mohamed Baz, Hanan Taie, Shawky Mustafa, and Hanem Khater. (2023): “Novel Acaricidal Efficacy of Nine Egyptian Plants against the Camel Tick, *Hyalomma Dromedarii* (Ixodida: Ixodidae).” *Persian Journal of Acarology* **12** (1): 121–36.
- Abeje, Fetene, Daniel Bisrat, Asrat Hailu, and Kaleab Asres. (2014): “Phytochemistry and Antileishmanial Activity of the Leaf Latex of *Aloe Calidophila* Reynolds.” *Phytotherapy Research* **28** (12): 1801–5.
- Adane, Agegnehu, Bogale Basaznew, Tesfaye Shimelis, and Dagnachew Shimelis. (2018): “Status of Mange Infestation in Indigenous Sheep and Goats and Their Control Practices in Wag-Himra Zone, Ethiopia.” *Journal of Veterinary Medicine and Animal Health* **10** (5): 128–34.
- Addisu, Dagne, Legesse Adane, and P Vijayalakshmi. (2016): “Phytochemical Investigation and Evaluation of Minimum Antibacterial Inhibitory Concentrations of Methanolic Extracts of the Root and Leaves of *Aloe Yavellana*.” *World Journal of Agricultural Sciences* **12** (3): 190–95.
- Adenubi, Olubukola T, Muna Ali Abdalla, Aroke S Ahmed, Emmanuel M Njoya, Lyndy J McGaw, Jacobus N Eloff, and Vinny Naidoo. (2018): “Isolation and Characterization of Two Acaricidal Compounds from *Calpurnia Aurea* Subsp. *Aurea* (Fabaceae) Leaf Extract.” *Experimental and Applied Acarology* **75** (3): 345–54.
- Adenubi, Olubukola T, Tlabo Leboho, Jacobus N Eloff, Gerda Fouche, Vinny Naidoo, Kevin W Wellington, and Lyndy J McGaw. (2019): “Acaricidal Activity of the Aqueous and Hydroethanolic Extracts of 15 South African Plants against *Rhipicephalus Turanicus* and Their Toxicity on Human Liver and Kidney Cells.” *Onderstepoort Journal of Veterinary Research* **86** (1): 1–7.
- Adugna, Hailemariam, and Habtamu Tamrat. (2022): “Epidemiological Study on Ixodid Tick Infestation and Tick Borne Haemopathogens on Cattle in Awi Zone, Northwest Ethiopia.” *Veterinary Medicine and Science* **8** (5): 2194–2205.
- Agegnehu, Hunegnaw. (2022): “Epidemiology and Accaricidal Efficacy Evaluation of Tick Infestation on Cattle In Selected Districts of Central Gondar Zone.”
- Arega, Melese, Aschalew Nardos, Asfaw Debella, Beyene Dereje, Lidet Terefe, and Abiy Abebe. (2023): “Evaluation of Anti-Inflammatory Activity of the Methanol Extracts of *Premna Schimperii* Engl (Lamiaceae) Leaves in Rats.” *Journal of Experimental Pharmacology*, 437–47.

- Argessa, Gemechu Duguma, Sena Megersa, Wondi Kabato, and Wabi Reggasa. (2025): "Phytochemical Analysis and Antimicrobial Activity of Carica Papaya Seed and Bark Against Selected Pathogens, in Case of Southern Ethiopia." *American Journal of Chemical Engineering* **13** (3): 66–75.
- Askale, G. (2015): "In Vitro Efficacy of Methanolic Extracts of Vernonia Amygdalina, Croton Macrostachyus, Ricinus Communis and Petroleum Ether Extract of Millettia Ferruginea against Bovicola Ovis and Rhipicephalus (Boophilus) Decoloratus." *Addis Ababa University, Ethiopia*.
- Bekele-Tesemma, Azene, Ann Birnie, and B Tengnas. (1993): "Useful Trees and Shrubs for Ethiopia." *Regional Soil Conservation Unit (RSCU), Swedish International Development Authority (SIDA)* **2**:123–27.
- Beyene, Sintayehu Tamene, Mesele Negash, Fortunatus Bulabo Bulabo, and Linley Chiwona Karlun. (2024): "Local Community Perception on Medicinal Plant Knowledge Use and Influencing Variables among Three Ethnic Groups in Peri-Urban Areas of South-Central Ethiopia." *Ethnobotany Research and Applications* **29**:1–16.
- Birhanu, Tadesse, and Dereje Abera. (2015): "Survey of Ethno-Veterinary Medicinal Plants at Selected Horro Gudurru Districts, Western Ethiopia." *African Journal of Plant Science* **9** (3): 185–92.
- Chatoui, K, A Talbaoui, M Aneb, Y Bakri, H Harhar, and M Tabyaoui. (2016): "Phytochemical Screening, Antioxidant and Antibacterial Activity of Lepidium Sativum Seeds from Morocco." *J Mater Environ Sci* **7** (8): 2938–46.
- Chatoui, Khalid, Hicham Harhar, Taha El Kamli, and Mohamed Tabyaoui. (2020): "Chemical Composition and Antioxidant Capacity of Lepidium Sativum Seeds from Four Regions of Morocco." *Evidence-Based Complementary and Alternative Medicine* **2020** (1): 7302727.
- Deshmukh, Mahendra A, and Madhuri A Theng. (2018): "Phytochemical Screening, Quantitative Analysis of Primary and Secondary Metabolites of Acacia Arabica Bark." *International Journal of Current Pharmaceutical Research* **10** (2): 35–37.
- Du, Yong-Hua, Ren-Yong Jia, Zhong-Qiong Yin, Zhong-Hui Pu, Jiao Chen, Fan Yang, Yu-Qun Zhang, and Yang Lu. (2008): "Acaricidal Activity of Extracts of Neem (Azadirachta Indica) Oil against the Larvae of the Rabbit Mite Sarcoptes Scabiei Var. Cuniculi in Vitro." *Veterinary Parasitology* **157** (1–2): 144–48.
- Dula, D E, and A Zelalem. (2018): "Phytochemical Screening of Calpurnia Aurea Root Extract." *Kenkyu Journal of Pharmacy Practice & Health Care* **4**:1997.
- Evans, Alec, Maxime Madder, Josephus Fourie, Lénaïg Halos, Bersissa Kumsa, Elikira Kimbita, Joseph Byaruhanga, Frank Norbert Mwiine, Dennis Muhanguzi, and Safiou Bienvenu Adehan. (2024): "Acaricide Resistance Status of Livestock Ticks from East and West Africa and in Vivo Efficacy of Acaricides to Control Them." *International Journal for Parasitology: Drugs and Drug Resistance* **25**:100541.
- Evans, W C. (2002): "Trease and Evans Pharmacognosy. 15th (Ed.) Saunders Publishers, an Imprint of Elsevier Science Ltd." India.

- Fatalo, Tesfaye, and Gebeyehu Alkadir. (2026): “In Vitro Acaricidal Activity of Croton Macrostachyus Leaf and Ricinus Communis Seed Extracts Against Cattle-Infesting Ticks (Rhipicephalus Spp. and Amblyomma Spp.).” *Veterinary Medicine International* 2026 (1): 1189650.
- Feyera, Tekla, and Edilu Abdisa. (2016): “In Vitro Acaricidal Activity of Crude Extracts of Schinus Molle (L.) Leaves against Field Population of Bophilus Decoloratus and Rhipicephalus Pulchellus Ticks.” *African Journal of Pharmacy and Pharmacology* **10** (36): 772–77.
- Fouche, Gerda, Bellonah M Sakong, Olubukola T Adenubi, Jean Paul Dzoyem, Vinny Naidoo, Tlabo Leboho, Kevin W Wellington, and Jacobus N Eloff. (2017): “Investigation of the Acaricidal Activity of the Acetone and Ethanol Extracts of 12 South African Plants against the Adult Ticks of Rhipicephalus Turanicus.” *The Onderstepoort Journal of Veterinary Research* **84** (1): 1523.
- Ghosh, SSRIKANTA, PALAVESAM Azhahianambi, and M P Yadav. (2007): “Upcoming and Future Strategies of Tick Control: A Review.” *Journal of Vector Borne Diseases* **44** (2): 79.
- Girma, Abayeneh, Indiris Abdu, Kasaye Teshome, Amere Genet, and Dessalew Tamir. (2024): ‘Prevalence, Trend Comparisons, and Identification of Ixodid Ticks (Acari: Ixodoidea) among Cattle in Ethiopia: A Systematic Review and Meta-Analysis.’ *Parasite Epidemiology and Control* 25:e00356.
- Habtemariam, Solomon. (2003) “In Vitro Antileishmanial Effects of Antibacterial Diterpenes from Two Ethiopian Premna Species: P. Schimperii and P. Oligotricha.” *BMC Pharmacology* **3** (1): 6.
- Hailesillassie, Tibebu, Daniel Bisrat, and Kaleab Asres. (201): “Larvicidal Effect of the Leaf Latex of Aloe Yavellana Reynolds and Its Major Compounds against Amblyomma Variegatum (Ixodidae).” *Veterinary Parasitology* **263**:23–26.
- Hankiso, Mulatu, Zemede Asfaw, Bikila Warkineh, Abiy Abebe, Bihonegn Sisay, and Asfaw Debella. (2024): “Ethnoveterinary Medicinal Plants and Their Utilization by the People of Soro District, Hadiya Zone, Southern Ethiopia.” *Journal of Ethnobiology and Ethnomedicine* **20** (1): 21.
- Heukelbach, Jorg, Richard Speare, and Deon Canyon. (2006): “Natural Products and Their Application to the Control of Head Lice: An Evidence-Based Review.”
- Holdsworth, P A, D Kemp, P Green, R J Peter, C De Bruin, N N Jonsson, T Letonja, S Rehbein, and Jozef Vercruysse. (2006): “World Association for the Advancement of Veterinary Parasitology (WAAVP) Guidelines for Evaluating the Efficacy of Acaricides against Ticks (Ixodidae) on Ruminants.” *Veterinary Parasitology* **136** (1): 29–43.
- Hunde Weyuma, Hunde Weyuma, Harpal Singh Harpal Singh, and Mulisa Megersa Mulisa Megersa. (2015): “Studies on Management Practices and Constraints of Back Yard Chicken Production in Selected Rural Areas of Bishoftu.”
- Isa, Mohammedamin Nasir, Wendimagegn Mammo, and Estifanos Ele Yaya. (2023):

- “Phytochemical Studies of *Premna Schimper*, and Antioxidant Activities of the Extract and an Isolated Compound.” *Bulletin of the Chemical Society of Ethiopia* **37** (6): 1471–86.
- Kaba, Tamirat. (2022): “Geographical Distribution of Ixodid Ticks and Tick-Borne Pathogens of Domestic Animals in Ethiopia: A Systematic Review.” *Parasites & Vectors* **15** (1): 108.
- Kemal, Jelalu, Yimer Muktar, and Sisay Alemu. (2016): “Distribution and Prevalence of Tick Infestation in Cattle in Babilie District, Eastern Ethiopia.” *Livest Res Rural Dev* **28** (12): 232.
- Kemal, Jelalu, Tesfaheywet Zerihun, Sisay Alemu, Kedir Sali, Musa Nasir, Ashebr Abraha, and Teka Feyera. (2020): “In Vitro Acaricidal Activity of Selected Medicinal Plants Traditionally Used against Ticks in Eastern Ethiopia.” *Journal of Parasitology Research* 2020 (1): 7834026.
- Krishnaveni, S, and P Venkatalakshmi. (2014): “S. Krishnaveni and P. Venkatalakshmi, ‘Antimicrobial, Larvi Cidal and Acaricidal Activities of the Ethanolic Extract of *Andropogon paniculatus* and *Carica papaya* Leaves,’ World Journal Pharmacology Research, Vol. **3**, Pp. 660–669, 2014.” *World Journal Pharmacology Research* 3:660–69.
- Kumar, Mandeep Kaur, Gurpreet Kaur, and Harleen Kaur. (2011): “Internationale Pharmaceutica Scientia.”
- Kumar, V, V Tomar, S A Ranade, H K Yadav, and M Srivastava. (2020) “Phytochemical, Antioxidant Investigations and Fatty Acid Composition of *Lepidium sativum* Seeds.” *J Environ Biol* **41** (1): 59–65.
- León, Adalberto A Pérez De, Robert D Mitchell III, and David W Watson. (2020): “Ectoparasites of Cattle.” *Veterinary Clinics of North America: Food Animal Practice* **36** (1): 173–85.
- Madzimure, James, Emmanuel T Nyahangare, Humphrey Hamudikuwanda, Thokozani Hove, Philip C Stevenson, Steve R Belmain, and Brighton M Mvumi. (2011): “Acaricidal Efficacy against Cattle Ticks and Acute Oral Toxicity of *Lippia javanica* (Burm F.) Spreng.” *Tropical Animal Health and Production* **43** (2): 481–89.
- Makwarela, Tsireledzo Goodwill, Nimmi Seoraj-Pillai, and Tshifhiwa Constance Nangammbi. (2025): “Tick Control Strategies: Critical Insights into Chemical, Biological, Physical, and Integrated Approaches for Effective Hard Tick Management.” *Veterinary Sciences* **12** (2): 114.
- Nath, Subhradal, Sanju Mandal, Susanta Pal, Suresh Jadhao, Namrata Ottalwar, and Prabir Sanyal. (2018): “Impact and Management of Acaricide Resistance: Pertaining to Sustainable Control of Ticks.” *International Journal of Livestock Research* **8** (10): 46–60.
- Nchu, Felix, Solomon R Magano, and Jacobus N Eloff. (2012): “In Vitro Anti-Tick Properties of the Essential Oil of *Tagetes minuta* L.(Asteraceae) on *Hyalomma rufipes* (Acari: Ixodidae).” *Onderstepoort Journal of Veterinary Research* **79** (1): 1–5.
- Negash, Nigatu, Dereje Andualem, and Belayhun Mandefro. (2024): “Phytochemical Screening and In-Vitro Efficacy of *Calpurnia aurea* against Two Transovarial Vectors: *Amblyomma variegatum* and *Rhipicephalus microplus*.” *Journal of Experimental Pharmacology*, 243–55.
- Nejash, Abdela Ahmed. (2016): “Bovine Babesiosis and Its Current Status in Ethiopia: A Review.”

Journal of Medicine, Physiology and Biophysics **23**:15–23.

- Onu, Simeon Haile, and Tesfaheywet Zeryehun Shiferaw. (2013): “Prevalence of Ectoparasite Infestations of Cattle in Bench Maji Zone, Southwest Ethiopia.” *Veterinary World* **6** (6): 291–94.
- Pirali-Kheirabadi, K H, and J A Teixeira da Silva. (2011): “In-Vitro Assessment of the Acaricidal Properties of Artemisia Annua and Zataria Multiflora Essential Oils to Control Cattle Ticks.” *Iranian Journal of Parasitology* **6** (1): 58.
- Qwarse, M. (2015): “Assessment of Bioactivity of Selected Plants against Pests and Microbes from Agro-Pastoral Communities in Mbulu District.” Open University of Tanzania.
- Razavi, Seyed Mostafa, Mohammad Asadpour, Arash Jafari, and Seyed Hossein Malekpour. (2015): “The Field Efficacy of Lepidium Latifolium and Zataria Multiflora Methanolic Extracts against Varroa Destructor.” *Parasitology Research* **114** (11): 4233–38.
- Regassa, AJJotSAVA. (2000): “The Use of Herbal Preparations for Tick Control in Western Ethiopia.” *Journal of the South African Veterinary Association* **71** (4): 240–43.
- Rocha, Sandra C, Clara Vásquez Velásquez, Ahmed Aquib, Aya Al-Nazal, and Nikhat Parveen. (2022): “Transmission Cycle of Tick-Borne Infections and Co-Infections, Animal Models and Diseases.” *Pathogens* **11** (11): 1309.
- Silva Lima, Elaine da, Diana Cuglovici Abrão, Douglas Luís Vieira, and Marcelo Beltrão Molento. (2022): “Allium Sativum Extract against Free-Living Larvae of Rhipicephalus Microplus: A Potential Environmental Biocontrol.” *Veterinary Parasitology: Regional Studies and Reports* **34**:100769.
- Sofowora, A. (1993): “Screening Plants for Bioactive Agents.” *Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria*, 134–56.
- Stafford III, Kirby C, Scott C Williams, and Goudarz Molaei. (2017): “Integrated Pest Management in Controlling Ticks and Tick-Associated Diseases.” *Journal of Integrated Pest Management* **8** (1): 28.
- Takele, WUBISHET. (2018): “Evaluation of the Antimicrobial Activities and Screening of the Phytochemical Composition of Crude Extracts from the Leaf Exudates of Endemic Aloe Species (A. Pirottae Berger and A. McLoughlinii Christian) in Eastern Ethiopia.” *Haramaya: Haramaya University.[Google Scholar]*.
- Tebabal Kebede, G. (2021): “Phytochemical Investigation, and Assessment of Antibacterial Activities of Calpurnia Aurea Root Bark.” *Nat. Prod. Chem. Res* **9**:383.
- Teka, Tekleab, Daniel Bisrat, Mariamawit Yonathan Yeshak, and Kaleab Asres. (2016): “Antimalarial Activity of the Chemical Constituents of the Leaf Latex of Aloe Pulcherrima Gilbert and Sebsebe.” *Molecules* **21** (11): 1415.
- Teklay, Abraha, Balcha Abera, and Mirutse Giday. (2013): “An Ethnobotanical Study of Medicinal Plants Used in Kilte Awulaelo District, Tigray Region of Ethiopia.” *Journal of Ethnobiology and Ethnomedicine* **9** (1): 65.

- Tesfaye, Tegegn, and Aschenaki Abate. (2023): “Knowledge, Attitude and Practices Study of Acaricide Usage and Tick Control in South Omo Zone Pastoral Areas, South-Western Ethiopia.” *Heliyon* **9** (6).
- Tesfaye, Tegegn, and Aschenaki, Abate. (2025): “In Vitro and in Vivo Screening of Commonly Used Acaricides against Ixodid Ticks in South Omo Pastoral Areas, South-Western Ethiopia.” *International Journal of Agricultural Research, Innovation and Technology (IJARIT)* **15** (1): 1–10.
- Vongkhamchanh, Bounthavy, Paweena Rattanasena, and Prapassorn Bussaman. (2014): “Acaricidal Activities of Crude Extract Derived from *Annona Squamosa* Linnaeus Leaves against Cattle Tick, *Rhipicephalus Microplus Canestrini* (Acari: Ixodidea).” *Journal of Science and Technology Mahasarakham University* **33** (2): 211–16.
- Zaman, Muhammad Arfan, Zafar Iqbal, Rao Zahid Abbas, Muhammad Nisar Khan, Ghulam Muhammad, Muhammad Younus, and Sibtain Ahmed. (2012): “In Vitro and in Vivo Acaricidal Activity of a Herbal Extract.” *Veterinary Parasitology* **186** (3–4): 431–36.
- Zorloni, A, Barend Louis Penzhorn, and Jacobus Nicolaas Eloff. (2010): “Extracts of *Calpurnia Aurea* Leaves from Southern Ethiopia Attract and Immobilise or Kill Ticks.” *Veterinary Parasitology* **168** (1–2): 160–64.

8. ANNEXES

Annex 1: Medicinal plant collection and wash with tap water



Annex 2: Authentication, Drying, and Grinding



Annex 3: Extraction, and Filtration

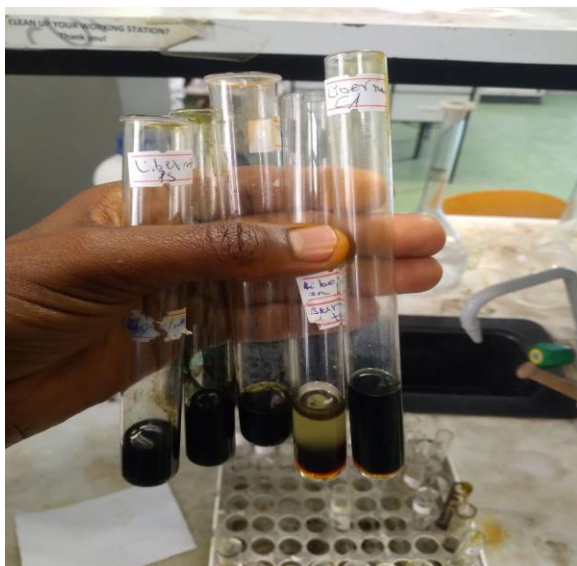
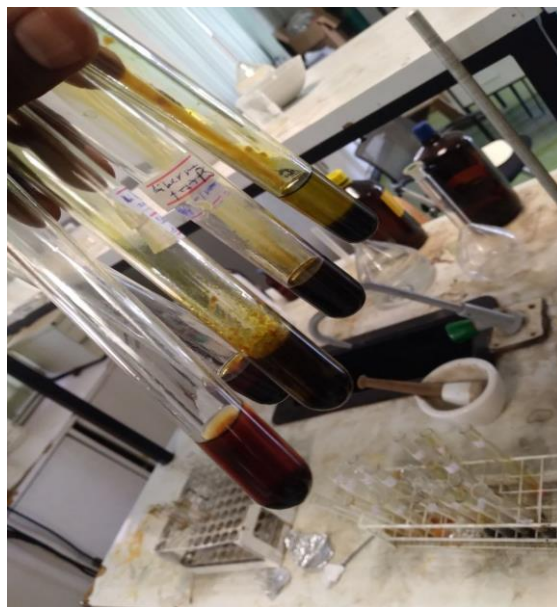


Annex 4: Acaricidal bioassay

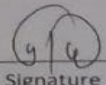


Annex 5: phytochemical test





Annex 6: Ethical clearance certificate

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Research Ethics Review Committee <i>Ethical clearance certificate</i>		
Certificate Ref. No: VM/ERC/09/110/18/2026		
Name of Applicant: Regassa Fidano Jima (DVM, MSc Student)		
Address: Department of Biomedical Sciences, College of Veterinary Medicine and Agriculture, Addis Ababa University		
Title of the project: <i>In vitro acaricidal activity of selected medicinal plants against ticks affecting domestic animals in Central Ethiopia</i>		
Date of application: November, 2025		
Nature of the project: Invitro efficacy study		
Target animal species: Cattle		
Number of animals involved: 150		
Study area: Central Ethiopia		
Minutes No. and date of review: VM/ERC/09/18/026, 26/03/2026		
The Institutional Animal Care and Use Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University has reviewed the above research project and unanimously approved the application of Student Regassa Fidano Jima.		
Professor Getachew Terefe (DVM, PhD) Chairman		 Signature
		
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ቢሾፍቱ፣ ኢትዮጵያ Bishoftu, Ethiopia		