Thesis Ref. No----------

ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

DEPARTMENT OF BIOMEDICAL SCIENCE

ASSESSING FARMERS' ACARICIDES USE AND EFFICACY EVALUATION OF DIFFERENT ACARICIDES AND SELECTED HERBAL EXTRACTS AGAINST CATTLE TICKS

MSc THESIS

BY

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JUNE, 2023

BISHOFTU, ETHIOPIA

ADDIS ABABA UNIVERSITY
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

ASSESSING FARMERS' ACARICIDES USE AND EFFICACY EVALUATION OF DIFFERENT ACARICIDES AND SELECTED HERBAL EXTRACTS AGAINST CATTLE TICKS

A thesis Submitted to School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirement for the degree of Master of Veterinary Pharmacology

By

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June, 2023

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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the Thesis prepared by **Solomon Desta** titled: "***Assessing Farmers' Acaricides Use and Efficacy Evaluation of Different Acaricides and Selected Herbal Extracts Against Cattle Ticks*****"** and recommend that it be accepted as fulfilling the thesis requirement for the degree of: Masters of Science in **Veterinary pharmacology**.

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Final approval and acceptance of the thesis is contingent upon the submission of its final copy to the CGS/FGC through the departmental graduate committee (DGC) of the candidate's major department. I hereby certify that I have read the revised version of this thesis prepared under my direction and recommend that it be accepted as fulfilling the thesis requirement.

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DEDICATION

This thesis manuscript work is dedicated to grace of God, and to my father, Desta Mishamo, and my mother, Askelach, Hafebo for nursing me with affection and love and for their dedicated partnership in the success of my life from primary school to MSc final thesis write-up.

STATEMENT OF AUTHOR

First, I declare that this thesis is my personal work and that all sources of material used for this thesis have been accordingly 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 truthfully declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate and no potential conflicts of interest with respect to the thesis authorship.

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ACKNOWLEDGEMENTS

First of all, I would like to thank the almighty God for his mercy and protection that give me health and strength throughout my work from the beginning up to now and forever.

My heartfelt thanks goes to the Gibe woreda Agricultural office for providing an opportunity to learn MSc in Veterinary Pharmacology.

I would like to express my heartfelt and gratitude to my advisors, Dr.Lishan Assefa, for his guidance, professional advice, and encouragement during my work.

Also, I would like to express my deepest respect and most sincere thankfulness to my co advisor, Takele Beyene (Associate Professor) for his guidance, and constructive comments of my work from the beginning up to the completion of this thesis.

I would like to thank the head of Graduate program, Prof. Bersissa Kumsa for his holistic guidance, advice and financial support.

My deep appreciation goes to Addis Ababa University, College of Veterinary Medicine and Agriculture, Department of Biomedical science and Ethiopian public health institute for allowing me to use laboratory and providing materials in my laboratory work.

Finally, My deep gratitude and grateful also goes to my family and all persons that have direct or indirect contribution for the accomplishment of this study.

LIST OF ABBREVATIONS AND ACRONYMS

AChE	Acetyl Choline Esterase
AIT	Adult Immersion Test
ANOVA	Analysis Of Variance
CP450	Cytochrome p450
CRD	Completely Randomized Design
CSA	Central Statistical Agency
CVMA	College of Veterinary Medicine and Agriculture
DDT	Dichlorodiphenyltrichloroethane
DMSO	Dimethyl Sulfoxide
EPHI	Ethiopian Public Health Institute
EVM	Ethnoveterinary Medicine
FAO	Food and Agriculture Organization
GABA	Gama Amino Butyric Acid
GC-MS	Gas Chromatography Mass-spectrometry
GPS	Global Positioning System
HSD	Honest Significance Difference
LC_{50}	Lethal Concentration
LCL	Lower Class Limit
NaOH	Sodium Hydroxide
OP	Organophosphates
OR	Odds Ratio
SE	Standard Error
SNNPR	Southern Nation Nationality People Region
TBD	Tick Born Disease

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ABSTRACT

Tick-controlling activity has been carried out by using a variety of commercially available chemical acaricides. However, the extensive use of chemicals promotes resistance and resulted in toxicity to animals and the environment, and residues in food animal products. Therefore, this study was aimed at assessing farmers' acaricides use and efficacy evaluation of different acaricides and selected herbal extracts against cattle ticks. The study was carried out using the questionnaire survey and in vitro experimental activity to evaluate the acaricidal activities of hydromethanolic leaf extracts of Calpurnia aurea, Datura stramonium, Nicotina tobaccum, and Ricinus communis against Amblyomma cohaerence by comparing with the efficacy of deltamethrin, diazinon, amitraz and five brands of ivermectin, and with DMSO (10%) as the negative control. Adult immersion test (AIT) following complete randomized design was used to test the efficacy. The result showed that farmers in the study area have poor knowledge (50%) and improper practices (91%) about acaricides use. Deltamethrin and the five brands of ivermectin were showed the highest level of efficacy (100±0.00 %) in tick mortality, while diazinon and amitraz were the least ranked (83.3±8.82% and 63.3±8.82%) within 72hrs of exposure time, respectively. There were statistical significance differences in efficacy between amitraz and other acaricides ($p = 0.000$). All extracts at the concentration of 100mg/ml were showed a significant difference in tick killing after 48hr post-exposure time, while below 50mg/ml showed insignificant effect ($p > 0.05$). At 72hr post-exposure time, N. tobaccum showed better efficacy (86.7±8.8%) followed by D. stramonium (76.7±6.7%) at 100mg/ml. Besides, R. communis showed slightly better efficacy (70±5.8%) than C. aurea with a statistical mean (63.3±8.8%). Finally, N. tobaccum and D. stramonium showed good acaricidal activity, followed by R. communis and C. aurea. While compared with commercial acaricides, all herbal extracts showed higher efficacy than amitraz 12.5%. Hence, the current study recommends, herbal extracts that showed high efficacy should be used as an alternative therapy and commercial acaricides with low efficacy should be reserved from using on animals to control tick infestation.

Key words**:** *Acaricides, A. cohaerence, Cattle, Efficacy test, Herbal extracts, Ticks.*

1. INTRODUCTION

Ethiopia has a large livestock population in Africa. However, the productivity is low, and ranks below average for most eastern and sub-Saharan countries, because of highly prevailing animal diseases like fungal, bacterial, viral and protozoan infection and tick infestation, poor nutritional status, reproductive insufficiency and lack of effective management (Bekele *et al*., 2010).

Ticks are considered the most damaging livestock pests on a global scale. In that, tick infestation and tick-borne diseases (TBD) are major constraints for a great diversity of livestock industry and health problems (Yilma *et al*., 2001). Ticks and tick- borne diseases are major causes of economic loss in the livestock sector in the tropics and subtropics (Mekuria, 1987) and throughout the world, which has tremendous economic importance in livestock production (Kettle, 1995). The problem is persistent and severe in developing countries where resources for control and eradication of ticks and TBD are limited (FAO, 1984). In most parts of Africa, including Ethiopia, tick and tick- borne diseases cause maximum economic loss due to livestock infestations, particularly affecting cattle (Solomon *et al*., 2001). In Ethiopia, at a conservative estimate, one million United States' Dollar (USD) is lost annually due to the rejection of hides and skin damaged by ticks (Bekele, 2002 and Gashaw, 2005).

In Ethiopia, several species of ticks have been reported including; the genus Amblyomma, Boophilus, Hyalomma, Haemaphysalis and Rhipicephalus, which are also vectors of transmits diseases like babesiosis, anaplasmosis, east coast fever and heart water (Walker *et al*., 2003). To mitigate this, over the past decades, ticks are mainly controlled by using a variety of commercial chemical acaricides; including organochlorines, organophosphates, carbamates, amidines and synthetic pyrethroids (George *et al*., 2004).

However, due to the genetic mutation of ticks to adapt to the extensive and improper use of different classes of acaricides, multiple resistance was developed over the ticks population, and reducing the efficacy of acaricides (Rodriguez-Vivas *et al*., 2011; Ayres

et al., 2013 and Corley *et al*., 2013). Under or over- concentration, widespread and frequent use of these chemicals might have also, led to the emergence of acaricide-resistant acarines in many countries (Dinka *et al*., 2013).

In addition to this, the use of commercial acaricides has resulted in environmental pollution, residuals in animal products, public health risks and the ever-increasing cost of acaricides (Graf *et al*., 2004). Hence, searching for indigenous knowledge-based natural products with effective acaricidal activity, particularly the use of medicinal plants has paramount importance to minimize the adverse effects of acaricides, overcoming the problems of resistance and for the healing of human and animal diseases due to the existence of certain specific substances, known as phytochemicals (Nostro *et al*., 2000).

Moreover, there is no finding reported on the efficacy evaluation of commercially available acaricides, specifically ivermectin and selected plant extracts against ticks of cattle in the Gibe district of the Hadiya Zone of the Southern region of Ethiopia. Their knowledge gaps in the use of appropriate dosage of acaricides were also not well documented. Therefore, this study was aimed at assessing farmers' acaricides use, practices and efficacy evaluation of different acaricides and selected herbal extracts against cattle ticks in the study area.

Specific objectives of the study:-

- To assess the knowledge and practices of farmers about acaricides use, residues and resistance
- To evaluate the efficacy of selected herbal extracts against cattle ticks
- To evaluate the efficacy commercially available acaricides in the study area
- To compare the acaricidal potential between selected herbal crude extracts and different brands of commercially available acaricides.

2. LITERATURE REVIEW

2.1.Ticks Biology

Ticks are ectoparasites under the phylum (Arthropoda), class (Arachnids) and the order Ixodidae (Acaridae) in the superfamily of Ixodes, with two major families, Argasidae (Soft mites) and Ixodidae (hard ticks). They are widespread throughout the world, especially in warm and humid climates, in that they feed on the blood of mammals, birds, and sometimes reptiles and amphibians as food. As such, they serve as vectors for many serious diseases that affect humans and other animals. They identify potential hosts by sensing odors, body temperature, humidity, and vibrations in their environment (Rodriguez-Vivas *et al*., 2004).

Adults have an ovoid/pear-shaped body (idiosoma) and eight legs with completely fused cephalothorax and abdomen. Hard ticks show clear sexual dimorphism with a frontal beak-like structure containing mouthpart and scutum, which covers the entire back in males and reduced to a small podonotal shield behind the capitulum in females and juveniles to allow great distention of the integument during feeding, whereas soft ticks have mouthparts on the underside of their bodies (Barker and Murrell, 2004; Rodriguez-Vivas *et al*., 2004).

Moreover, ticks in the Ixodidae family are relatively large and include certain species of veterinary and medical importance, like Amblyomma, sub genus Rhi.(Boophilus), Rhipicephalus, Haemaphysalis, Hyalomma, Dermacentor and Ixodes. The Argasidae or soft ticks have Ornithodoros which is an important genus that affects cattle. An adult argasids lack a sclerotic back plate or scutum. In that, the skin is leathery and wrinkled, the mouthparts are not visible from above, and they showed no apparent sexual dimorphism. Besides, it is a migratory tick that stays on its host only while it feeds (Nejash, 2016).

Ticks have four life cycles: eggs, larvae, nymph and adults. Hard ticks occupy a one-host, two-host, or three-host lifestyle. But, soft ticks have multiple life cycle which is up to seven nymph stages (instars), each requiring blood sampling (Kirby, 2010).

2.2. Prevalence of Ticks in Ethiopia

Research on tick fauna began in Ethiopia in the early 19th century. Since then, various foreign and domestic researchers have determined pattern of ticks and TBDs. Ticks are common in all agroecological zones of the country (Nejash, 2016). Among these, the incidence of vectors and vector-borne diseases is prevalent in tropical and subtropical regions of countries where huge economic activities depend on livestock (Asefa *et al*., 2017).

A study in Ethiopia showed that approximately 75.7% of livestock were infested in 50% expected prevalence by one or more tick species. The average prevalence of each genera was Amblyomma (43.7%), Rhipicephalus (Boophilus) (20%), Rhipicephalus (17.7%) and Hyalomma (9.6%). The study also showed that the prevalence of tick infestations was higher in older and poor body-conditioned animals (Jelalu *et al*., 2016; Asefa *et al*., 2017; Bereket and Tekalign, 2019; Hordofa *et al*., 2021).

Amblyomma was the most dominant genus of ticks and Hyaloma was the least genus in various studies. Among the identified species, *B. decolorotus* was the most common species in this study, followed by *A. verigatum*, *R. evertsi evertsi*, *H. marginatum, A. lepidum, and A. cohaerence* (Bereket and Tekalign, 2019). The prevalence of tick infestation depends on various factors such as breed, age, sex, and body condition. The prevalence of tick-infected cattle was higher in crossbreds than in local breeds (Hordofa *et al*., 2021).

In general, the major tick genera found in the cattle population of Ethiopia are Amblyomma, Rhipicephalus (Boophilus), Rhipicephalus, and Hyalomma (Walker *et al*., 2003, Sileshi *et al*., 2007). The genus Ambylomma and Rhipicephalus (Boophilus) are

predominating in many parts of the country; Hyalomma and subgenus Rhipicephalus also have a significant role in infestation of livestock (Solomon *et al*., 2001).

2.3. Economic Impacts of ticks

Ethiopia is considered to have the highest number of cattle in Africa. This livestock sector plays an important role in the country's economy and still promises enormous economic development for the country (CSA, 2013). That represents a major asset among resource-poor smallholder farmers by providing milk, meat, skin, manure, and traction force (Mesfin and Lemma, 2001). In addition, animal husbandry contributes to the national economy, particularly in terms of foreign exchange income, through the exploration of live animals, meat, hides and skins (MoARD, 2008).

However, poor health and productivity of animals due to disease have considerably become the major stumbling block to the potential of the livestock industry (Mekonnen *et al*., 2001). Particularly, ticks transmit a large variety of intercellular bacteria in the Rickettsia group like Rickettsia, Ehrlichia and Anaplasma. Similarly, several piroplasm protozoa like B. *bigemmina*, T. *annulata* and T. *parva* are also transmitted specifically by ticks. These cause the most important tick-borne diseases like East Coast Fever, Redwater, babesiosis, theileriosis, anaplasmosis and heartwater. Some of them are fatal and benign like babesiosis, theileriosis and anaplasmosis (Jongejan and Uilenberg, 2004).

These disorders commonly affect the blood and/or lymphatic system and can lead to fever, anemia, jaundice, anorexia, weight loss, decreased milk production, swollen lymph nodes, difficulty in breath, diarrhea, nervous disorders, and even death (Nejash, 2016). Severe irritation and trauma, the introduction of toxins and damage to animal hides and skin can lead to animal mortality, reduced productivity and fertility, deterioration and rejection of hides and skins which causing great economic losses to smallholder farms, the tanning industry and the entire country (Rajput *et al*., 2006; Marufu, 2008; Tiki and Addis, 2011). Due to the economic and veterinary importance, control of ticks and

transmission of tick-borne diseases remains a challenge for animal husbandry worldwide, especially, in tropical and subtropical regions (Lodos *et al.,* 2000).

All these factors impact the great diversity of livestock health problems. Approximately 80 % of the cattle population in the world faced a risk of tick infestation and tick-borne diseases which inflicts huge economic loss that is estimated at 13.9 to 18.7 billion USD annually (Ghosh *et al*., 2007). The global costs required for tick control and productivity losses account for 700 million USD annually (Yilma *et al*., 2001). In Ethiopia, an annual conservative estimated loss of 1 million USD is attributed to the downgrading of hides and skin due to tick infestation. Moreover, light to severe inflammatory reactions sometimes leading to surgical removal of teats are damages caused by ticks (Yilma *et al*., 1995; Nejash, 2016). From this, only eastern Ethiopia lost 500,000 USD from hide and skin downgrading, and approximately 65.5% of the major defects of hides were due to ticks (Bekele, 2002).

Furthermore, with the inclusion of losses from reduced productivity, deaths and costs of tick control, the estimated total loss will be much greater than this. The comparative data on the economic losses from ticks and tick-borne diseases in African livestock are missing. However, an estimated 168 million USD in Eastern, Central and Southern Africa is caused only by East Coast Fever (Dipeolu *et al*., 1992). These losses are exacerbated in tropical and sub-tropical environments where the host is subjected to stresses associated with sub-optimal nutrition and high environmental temperature. In addition to this, the costs associated with maintaining chemical control of ticks in tropical and subtropical regions of the world have been estimated at 25.00 USD per head of cattle per year (Pegram, 2001).

2.4. Methods of Tick Control in Ethiopia

Successfully implementing rational and sustainable tick control programs in the area where livestock is produced depends on a sound knowledge of the ecology or epidemiology of the tick in specific climatic environments. However, most of the time

efficient and reliable methods for controlling cattle ticks and TBD depend on using acaricides without understanding suitable ecology or epidemiology. Even, though the use of acaricide is the most efficient and reliable single method, complementary approaches have been developed and are being researched to enable integrated control strategies against the tick and its haemoparasites (Alanr, 2011).

It is essential to evaluate the availability of each of these options as well as the advantages and disadvantages of each control program before selecting an action that targets the parasitic and free-living phases of the life cycle as well as the role that ticks play in the transmission of TBDs (Kirby, 2010). The tick control methods that are most frequently used in various parts of the country are briefly described below:

2.4.1. Genetic tick control

The use of acaricides to control ticks in the cattle population has its own drawback. Therefore, the production of various breeds of cattle with the potential to be genetically resistant to ticks can contribute to the creation of alternative methods of control to mitigate this issue. As a result, Bos indicus cattle are more resistant to ectoparasites than Bos taurus cattle. The crossing of these two breeds has also produced high-quality meat and increased resistance to the environment (Bianchin *et al*., 2007).

2.4.2. Biological tick control

The introduction of tick parasitic agents such as wasps, birds, parasitoids, entomo pathogenic nematodes, entomo pathogenic fungi, and bacteria is the primary focus of the biological tick control method (George *et al*., 2008). In addition, by consuming a large number of ticks, predators such as rodents, birds, ants, spiders, lizards, and beetles play a significant role in tick control (Latif and Walker, 2004). The wasp lays eggs in the engorged ticks, eats the tick's larvae, which then emerge as an adult to attack another tick, and parasites (Nematodes and fungi) attack the ticks' soil-living stages (Kirby, 2010).

2.4.3. Ecological tick control

An ecological control method is used for habitat and host-linked treatment. Tick control in the habitat and vegetation requires modification of the plant cover by removal of vegetation that shelters ticks (Kirby, 2010). Pasture management and seasonal changes in cattle grazing areas have been used as a tick control strategy and are believed to be responsible for a decrease in its burden (Walker *et al*., 2003).

2.4.4. Chemical-based tick control

During times of high tick infestation, acaricide treatments are frequently applied in a suppressive manner, applying multiple treatments on a regular basis. In the short term, suppressive treatments are the most effective; keeping animals nearly tick-free, thereby lowering the likelihood of disease transmission and the direct effects of ticks. However, this method will disproportionately target ticks with acaricide resistance (George *et al*., 2008).

An ideal acaricide would be inexpensive, easy to apply, have a strong knockdown effect, be safe for humans and livestock, and have enough residual effect on female ticks to stop them from laying eggs and keep cattle from being re-infested by larvae and leave no traces in milk or meat. It should have a sharp cut-off in efficacy with time and not select for resistance through a prolonged, gradual decay on the animal. Inappropriately, no such ideal acaricide has yet been developed. Tick resistance to acaricides is a growing issue and a real economic threat to livestock and the use of acaricides for tick control generally has limitations. Besides, livestock owners rely solely on acaricides to control ticks. However, they do not have access to guidelines on how to profit from ticks or how to identify and address issues with resistance to acaricides (Nejash, 2016).

2.5. Development of Acaricides Resistance in ticks

Resistance is generally first recognized as the failure of a drug to control parasitism, but the formal definition of resistance is a shift in the target species' susceptibility to a drug

(Corley *et al*., 2013). According to the world health organization scientific group (WHO, 1965), resistance is "the ability of a parasite strain to survive or to multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended. There are three types of acaricidal resistance. These are acquired resistance, cross-resistance, and multiple resistances (Abbas *et al*., 2014).

Acquired resistance refers to resistance that inherits the decreasing activity of drugs over time (Meyer *et al*., 2012). A decrease in the population of ticks that are susceptible to an acaricide is accompanied by an increase in resistant strains due to a direct relationship between the concentration of the drug, continued exposure, and degree of acaricide resistance (Faza *et al*., 2013).

Cross-resistance is the sharing of resistance among different acaricides with a similar mode of action, like two organophosphates groups (coumaphos and diazinon) and one carbamate (carbaryl) acaricides in several strains of *R. microplus* (Abbas *et al*., 2014; Perez-Gonzalez *et al*., 2014). These two acaricides (organophosphates and carbamates) exert their toxic effects on ticks by inhibiting acetylcholinesterase (AChE), which gives function to the nervous system of invertebrates (Jensen *et al*., 2011). The resistance of ticks against carbamates and organophosphates is developed by reducing sensitivity to a mechanism of action of AChE (Dawkar *et al*., 2013).

Multiple resistances are resistance to more than one therapeutic agent, even if they have different modes of action. Nowadays, it is a threat to the chemical control of cattle ticks. In the tick population, multiple resistances against acaricides with different modes of action lead to suspect that resistance may be metabolic (Bielza *et al*., 2007).

2.6. Mechanism of Acaricides Resistance

The first development of resistance of *B. microplus* and *B. decoloratus* to arsenic was reported from Australia and South Africa respectively and reports of amitraz resistance to Boophilus spp in different parts of the world. The emergence of resistance to a variety of

acaricides like organophosphate, pyrethroid, formamidine, and macrocyclic lactone acaricides in both single and multi-host ticks was an indication of the gradual development of resistance (George *et al*., 2004).

In addition to this, macrocyclic lactone resistance of *B. microplus* was reported in Brazil, especially to doramectin with cross-resistance of ivermectin. Widespread use of arsenic, chlorinated hydrocarbons, organophosphates, carbamates, pyrethroids, and macrocyclic lactone for tick control has caused major concern for the development of resistance (Martins and Furlong, 2001). Therefore, understanding basic knowledge of the mode of action of acaricides is very important to know the mechanisms involved in the development of resistance.

2.6.1. Resistance against organochlorine

Organochlorine was the first synthetic insecticide to be marketed and has been formulated for the control of ticks on cattle since 1946. The mode of action of these compounds is binding at the picrotoxinin site in the γ-aminobutyric acid (GABA) chloride ionophore complex (Hope *et al*., 2010) which inhibits Cl^- flux into the nerve (Corley *et al*., 2012). The function of the organochlorine with GABA-ergic inhibitory neurons is impaired and hyperexcitation results in death. The mechanism of resistance was preceded by boosting metabolism and reducing the absorption of the chemical (George *et al*., 2004; Abbas *et al*., 2014).

2.6.2. Resistance against amidines (Amitraz)

Amitraz is a member of the amidine class and has been used as an effective treatment against the ticks of cattle for more than 45 years (Jonsson and Hope, 2007), but resistance has been reported (Mendes *et al*., 2013; Abbas *et al*., 2014). The mode of action of amitraz is by inducing its toxic effects on a receptor for the neuromodulator (octopamine), which contains two nucleotide substitution molecules on the resistant strains of ticks that result in amino acids different from all the susceptible strains (Corley *et al*., 2013).

In that, it is synergistically involved with P450 cytochrome monooxygenases by modification of the target site (Gong *et al*., 2013). The Discovery of these mutations in amitraz-resistant ticks provided the first evidence for the possibility of an altered target site as a mechanism of amitraz resistance in ticks. However, the exact mechanism of resistance to amitraz is not well described (Guerrero *et al*., 2012).

2.6.3. Resistance against pyrethrins (Pyrethroids)

Pyrethrins are naturally occurring compounds derived from flowering plants called the chrysanthemum family. They have a quick knock-down effect against arachnids, but they are unstable in the environment and do not persist actively long enough to kill ticks. Pyrethroids are the synthetic version of pyrethrins, which are designed to be more stable than the pyrethrums and to achieve a longer-lasting effect. Both pyrethrins and pyrethroids are potent neurotoxins. They act on sodium ion channels and thus cause nerve excitation due to changes in nerve membrane permeability to sodium and potassium ions (Weston *et al*., 2013; Abbas *et al*., 2014).

Voltage-gated sodium channels are the target of pyrethroid activity and resistance development in a wide range of pests and disease vectors (Guerrero *et al*., 2012). The involvement of p450s (Chevillon *et al*., 2007) and esterases (Li *et al*., 2013) in pyrethroid resistance has been demonstrated for many species of ticks (Abbas *et al*., 2014) by developing a mutation that decreases the channel sensitivity to pyrethroids (Oliveira *et al*., 2013).

2.6.4. Resistance against organophosphates

The chronological order of acaricidal development started with arsenic, which was the first effective acaricide used for controlling ticks, and tick-borne diseases in different parts of the world before resistance developed. After resistance developed, this chemical was replaced by chlorinated hydrocarbons (DDT), which have been highly persistent in the environment and used extensively throughout the world for controlling ticks (Rajput *et al*., 2006).

Organophosphates (OPs) were introduced around 1950, to replace chlorinated hydrocarbons, which have a wide range of activities against ticks at very low concentrations and developed significant resistance. Among the first chemical groups used to control arachnids, OPs were the leading one. The mechanism of action of OPs was exerting their toxic effects on ticks by inhibiting acetylcholine esterase (AChE), which is a key enzyme to the functioning nervous system (Faza *et al*., 2013; Temeyer *et al*., 2013a, b).

When ticks are poisoned with a cholinesterase inhibitor, the cholinesterase is not available to help break down the acetylcholine, and the neurotransmitter continues to cause the neuron to "fire" or send its electrical charge. This results in overstimulation of the nervous system and the death of the arachnid. But, the mechanism of resistance for Ops can be developed by increasing the activity of AChE due to mutations in AChE genes and oxidative metabolism (Van Leeuwen *et al*., 2009; Lwande *et al*., 2012).

2.6.5. Resistance against macrocyclic lactones

Macrocyclic lactone acaricides include avermectins and milbemycins, which are naturally occurring products of actinomycetes (genus Streptomyces) by the process of fermentation. Milbemycin was first derived from a culture of Streptomyces hygroscopicus and is structurally similar to the avermectins but lacks the disaccharide at C13 (Takiguchi *et al*., 1980) and was first reported as the effective acaricidal agent by (Mishima *et al*., 1975).

It blocks the transmittance of electrical activity in nerves and muscle cells by stimulating the release and binding of gamma-aminobutyric acid (GABA) at nerve endings (Martin *et al*., 2012). This causes an influx of chloride ions into the cells leading to hyper polarization and subsequent paralysis of the neuromuscular systems (Abbas *et al*., 2014). The mechanism of resistance in nematodes against macro cyclic lactones is due to target site insensitivity of the GABA or glutamate-gated chloride ion channels (Lovis *et al*., 2013).

2.7. Risk Factors in Resistance Development of Ticks

Populations of ticks, especially *B. microplus*, developed resistance to many classes of acaricide, including chlorinated hydrocarbons (DDT), pyrethroids, organophosphates, and formamidines (amitraz). This resistance development in the tick population is associated with three factors. First, the genetic factors of parasites include the dominance of resistance alleles, the number of genes involved, the genetic diversity of the population, the relative fitness of resistance, and genetic recombination (Abbas *et al*., 2014).

Second, biological aspects are mainly associated with the host–parasite relationship and mechanism of selection for resistance. For example, parasites that induce effective immunity in their hosts have weaker selection pressure for resistance because immunity selects parasites regardless of drug-resistance status, and this reduces the chance of parasites surviving and reproducing (Abdullah *et al*., 2012).

Third, operational factors include the chemical nature of the drug, drug persistence in the host, application and selection threshold, life stages selected, mode of application, frequency of treatment, frequent use of the same acaricide for a long period of time, under-dosing, and poor drug quality (Rezende *et al*., 2013). The use of acaricide greater than five treatments per season is a positive risk factor for acaricide resistance (Jonsson *et al*., 2000).

2.8. Strategies to Minimize Acaricidal Resistance

A well-understanding of tick population dynamics and their survival in diverse natural habitats might be essential to initiate the formulation of integrated, suitable, efficient, and economic tick control measures (Luciana *et al*., 2011). Besides, information on the status and magnitude of acaricide resistance is vital in deciding the appropriate tick and tick-borne disease control strategy in different localities in Ethiopia (Gebre *et al*., 2004).

There are a few options used to reduce resistance, by diagnosing the evolution of acaricide-resistant tick, in that acaricides become ineffective in control programs (George *et al*., 2004). These include the rationale use of acaricides by means of monitoring (Jonsson *et al*., 2000, Sugimoto and Osakabe, 2013), the use of combinations (mixtures) of acaricides (Lovis *et al*., 2013), using vaccination to enhance immunity in cattle (Freeman *et al*., 2010), improving nutritional management (Wikel, 2013), and improving genetic resistance in cattle (Ayres *et al*., 2013; Rodriguez-Valle *et al*., 2013).

Besides, rotation or alternation of acaricides having different modes of action, environmental management like pasture burning, pasture alternation or rotation, house management, and development of effective diagnostic techniques are used as ways of tick resistance mitigation options (Abbas *et al*., 2014).

2.9.Herbal Medicine Based Tick Control

In different parts of the country, tick control depends on the use of acaricides like toxaphene, dieldrin, amitraz, diazinon, and ivermectin. However, the improper use of these chemicals has been causing the development of tick resistance to various pesticides and reducing the lifetimes of these products. Besides, problems generated by the presence of chemical residues in meat, milk, and the environment have prompted better monitoring of their application (Castro-Janer *et al*., 2010).

Because of the hard delayed degradation, their residues usually remain in agricultural environments where they adversely affect the life of living organisms in the natural ecosystem (Habeeb, 2010). This has led to environmental pollution, the development of resistant tick strains, escalating costs, and public health risk (Dinka *et al*., 2013). For this reason, there is a renewed interest in botanicals for safe, effective, and cheap control of ticks (Babar *et al*., 2012). The application of botanicals to control ectoparasites of veterinary importance is a widespread practice in developing countries (Zaman *et al*., 2012).

In Ethiopia, medicinal plants and the knowledge of how to use them are crucial to the health of people and livestock. The country's healthcare options show that traditional medicine is used by 70% of humans and 90% of livestock (Abebe *et al*., 2003). In this way, plant extracts have been widely used by livestock producers to control ticks in rural areas where cultural practices are still in place and because of limited financial resources (Liang *et al*., 2003). This practice is typically community-based, and the plant species used for such purposes may vary from one community to another. The knowledge of such practice is orally transferred from one generation to another and often lacks scientific validation (Sisay and Jelalu, 2015).

2.10. Phytochemical Treatment

Even if, the uses of ethnoveterinary medicine (EVM) probably reduce tick-burdens while maintaining endemic stability to tick-borne diseases little work has been done to document and validate these EVM in Ethiopia (Regassa, 2000). Furthermore, the efficacy of most plants that have been traditionally used hasn't been scientifically tested. Due to the economic and medical importance of ticks, it is necessary to screen some ethnoveterinary plants that have acaricidal properties (Matlebyane *et al*., 2010). Plant-derived substances have recently become of great interest in phytochemical therapy. Phytochemicals are the richest bio-resource of drugs of traditional medicine, modern medicines, folk medicines, food supplements, nutraceuticals, pharmaceutical intermediates, and chemical entities for synthetic drugs (Ncube *et al*., 2008).

They still offer a huge potential supply of new chemotherapeutic agents and served as the foundation for the treatment of various diseases. Animals and humans have been treated for ectoparasites using various plant parts like roots, barks, leaves, seeds, stems, roots, flowers, and fruits (Tiwari *et al*., 2011; Teklay and others, 2013). The following are plants selected for this study based on their traditional use by farming communities.

2.10.1. Calpurnia aurea

Calpurnia aurea is a plant, a member of the family Fabaceae. It is a small, multi-stemmed tree, 3–4 m tall, and widely distributed throughout bush land, and grass land, in tropical and sub-Saharan Africa and India. In southern Ethiopia, it is called Senna by the Hadiya people and enjoys a number of ethnomedicinal uses to treat diverse medical conditions and parasitic infestation, both in humans and animals. Traditionally, the leaves are used to treat syphilis, malaria, rabies, diabetes, hypertension, leishmaniasis, trachoma, elephantiasis, fungal diseases, stomachache, bowel, and bladder disorders, and different swellings. It also possesses good antidiarrheal and antimicrobial activity (Umer *et al*., 2013).

Moreover, the extract of leaves, powdered roots, and barks are used to destroy lice and maggot in wound and louse infestations in humans and calves and for tick control as an insecticide (Regassa, 2000), and its seed is also used to treat and relieve allergic rashes, itches, bacterial dermatitis, abscesses, stomach complaints, headache, earache, rheumatism, antioxidant and eye diseases, scabies, and skin infection caused by ticks (Asres *et al*., 2001). The acetone, hexane, and water extracts of the leaf of this plant are used to protect stock against ticks. The aqueous solution has effective acaricidal properties on adult *R. pulchellus* (Sisay and Jelalu, 2015).

2.10.2. Ricinus communis

The plant is known as 'the castor plant or the palm of Christ and is locally called Qoboo in the Hadiya language. It is found in the Family of Euphorbiaceae. It is a small fast-growing suckering perennial shrub or soft-wooded small tree up to 6 meters or more, and is widespread throughout tropical regions and all over India. All parts of the plant (bark, leaves, flowers, seed, and oil) are important for different pharmacological activities. This plant was cultivated for leaf, and flower colors and for oil production. The leaves are green or reddish in color and about 30-60 cm in diameter (Jena *et al*., 2012).

Traditionally, the plant is used as fertilizer, fungicide, anti-oxidant, antihistaminic, antinociceptive, antiasthmatic, antiulcer, antitumor immunomodulatory, antidiabetic, hepatoprotective, antifertility or anti-implantation, anti-inflammatory and free radical scavenging activity, antimicrobial, central nervous system stimulant (central analgesic), lipolytic, wound healing, insecticidal and larvicidal. Besides, its oil is widely used in Ayurveda, a homeopathic and allopathic system of medicines as cathartic (laxative and purgative) (Rana *et al*., 2012).

Phytochemical screening of leaf extracts of *R. communis* contains saponins, tannins, phenolic compounds, steroids, flavonoids, glycosides, triterpenes, phlobatannin, and alkaloids. The high toxicity of *R. communis* extracts against ticks was due to the presence of these bioactive. Methanolic extract of the leaf has good acaricidal activity against *Rh. Pulchellus* even at lower concentrations (Jelalu *et al*., 2020). In addition, the vitro and in vivo activity of the extract of leaves possess high acaricidal properties, which may provide an effective herbal formulation for the control of multi-acaricide-resistant tick infestation on animals (Ghosh *et al*., 2013).

2.10.3. Nicotina tobaccum

Tobacco is a cultivated herbaceous plant of the genus Nicotiana under the family of Solanaceae that is grown annually in the tropics and throughout the world. It is locally called Tambaa in the Hadiya language. It grows to heights between 1 and 2 meters (Ren *et al*., 2001). The outstanding feature of the tobacco plant is the extensive leaf area used in cigar manufacture as nicotine. The leaves of *N. tobacco* have acaricidal and insecticidal efficacy in the treatment and prevention of lice and pesticidal agents against *B. microplus* (Iqbal *et al*., 2006). It kills larvae and nymphs of *R. appendiculatus* on the ears of calves and large numbers of adult ticks in vitro after a few hours of application (Sisay and Jelalu, 2015).

The literature shows tobacco leaves are contained triterpenoids, alkaloids, glycosides, saponins, flavonoids, tannins, and phenols, which are responsible for health benefits such as antibacterial, antifungal, anthelmintic, antinociceptive, anti-Alzheimer's activities and

bioinsecticide. In addition, *Nicotina tabucum* has a pharmacological role in abdominal discomfort, constipation, urinary tract obstruction, dental pain, gastrointestinal disorders, and dermatitis (Oyekunle *et al*., 2019; Yati *et al*., 2022). The phenolic and its derivate exhibit free radical inhibition, peroxide decomposition, metal inactivation, or oxygen scavenging in biological systems and prevent oxidative disease burden. However, there is no toxicity, no mortality, and no significant change in behavior and body weight against female winstar rats at a dosage of 5000 mg/kg (Yati *et al*., 2022).

2.10.4. Datura stramonium

Datura stramonium is stinking and a branched herbaceous, leafy, flowering annual plant under the taxonomic family of Solanaceae that is extensively distributed in the temperate and tropical regions of the globe and native to Central America. The plant is commonly known as jimson weed, green thorn apple, Jamestown weed, or devil's apple and is also locally called Macharra in the Hadiya language which, grows in sandy flats, plains, and areas up to 2,500 feet above sea level (Singh *et al*., 2013).

It has oval-shaped leaves, which has 10–20 cm long, 5–18 cm wide and coarsely dentate on the margins. They are smooth and jagged, with darker green on the upper surface and light green on the lower surface (Shyma *et al*., 2014). The trumpet-shaped, erect, or spreading flowers are white to yellow, pink, and pale purple in color and measure 5 to 20 cm long and 4 to 12 cm wide at the opening. The fruit is a large, oval, spiny capsule that splits open into four chambers when ripe for release and contains numerous seeds that range in color from black to dark brown (FAO and WHO, 2020).

The diverse biological activity of extracts of *Datura stramonium* includes antibacterial, antifungal, anti-inflammatory, larvacidal, antispasmodic, antinociceptive, antioxidant, hypolipidemic, anti-rheumatoid, and hypoglycemic properties because of chemical compounds like tropane alkaloids, amino acids, tannin, phytic acids, and carbohydrates which have been isolated. Moreover, the dried leaves, roots, or flowers are used to cure asthma, cough, tuberculosis, bronchitis, swellings, wounds, gout, burns, ingrown toenails,

fungal infections, tumors, and ulcers. The cigarettes made with the leaves are also used to treat Parkinson's disease (Singh, *et al*., 2013).

In addition to this, the leaf extracts have significant acaricidal activities and inhibition of oviposition against engorged female ticks of *B. microplus* and *B. decolrtus* (Demisse and Wgebrial, 2018). Even if, they are used for pharmacological purposes, considered to be poisonous weeds because of high amounts of tropane alkaloid, which is the anticholinergic substance that causes a variety of adverse effects including mortalities in humans and animals due to accidental ingestion (FAO and WHO, 2020).

2.11. General Methods of Extraction of Medicinal Plants

As a result, it is essential to establish the scientific foundation for the therapeutic effects of medicinal plants, because these plants may provide the basis for the development of more effective drugs. The standard procedures for extracting crude drugs from parts of plants aim to remove unwanted material through the use of a selective solvent known as the menstruum and reach the therapeutically desired portions. Alkaloids, glycosides, terpenoids, flavonoids, and lignans are just a few of the medicinal plant metabolites found in these products, which make up a complex mixture. The most common extraction methods are maceration, infusion, percolation, digestion, and alcoholic fermentation (Handa *et al*., 2008).

2.11.1. Maceration

In this method, the whole or coarsely powdered crude drug, which can be leaves, stem bark, or root bark, is put in a container with a stopper. The solvent (menstruum) is poured on top until it completely covers the drug and is left at room temperature for at least three days. During this time, the container is frequently shaken to make sure the soluble matter has dissolved. After that, the mixture is strained; the marc, which is the damp solid material, is pressed, and the combined liquids are clarified through filtration or decantation after they have been stood. After that, either in an oven or on top of a water

bath, the micelle is evaporated to separate from the menstruum. This method is very suitable for thermo-labile plant material and is convenient (Ingle *et al*., 2017).

2.11.2. Soxhlet extraction.

This method of continuous hot extraction makes use of a glass apparatus known as a Soxhlet extractor. It has a condenser at the top, a siphon tube, an extraction chamber, and a flask with a round bottom. The crude drug is finely ground and placed in a porous bag or "thimble" made of clean cloth or strong filter paper in a closed Soxhlet chamber (Hossain *et al*., 2014). The bottom flask is filled with the extraction solvent, and the thimble is poured into the extraction chamber. The dissolvable is then warmed from the base flagon, dissipates, and goes through the condenser where it consolidates and streams down to the extraction chamber and concentrates the medication by coming in touch. The solvent and the extracted plant material flow back to the flask when the level of solvent in the extraction chamber reaches the top of the siphon (Ingle *et al*., 2017).

3. MATERIALS AND METHODS

3.1.Description of the study area

The study was conducted in Gibe district, one of the 13 districts of the Hadiya zone, Southern Nations nationality people regional state /SNNPRS/ of Ethiopia (Figure 1). It is situated 262 Km south of Addis Ababa and 32 Km South West of Hosanna city. Geographically it lies at 7^0 37'53" -7^0 42'43''N Latitude and 37^0 37' 7''-37^0 44' 25'' E Longitudes. According to the local agro-climatic classifications, the district has Kola, Woynedega, and Dega climatic characteristics, with the annual range of rainfall and temperature from 600 to 1200mm and 17.6 to 25 ^{0}C, respectively. The district has a large population of livestock which needs supervision, proper management, health care and sufficient laboratory investigation of diseases, for further production and disease control.

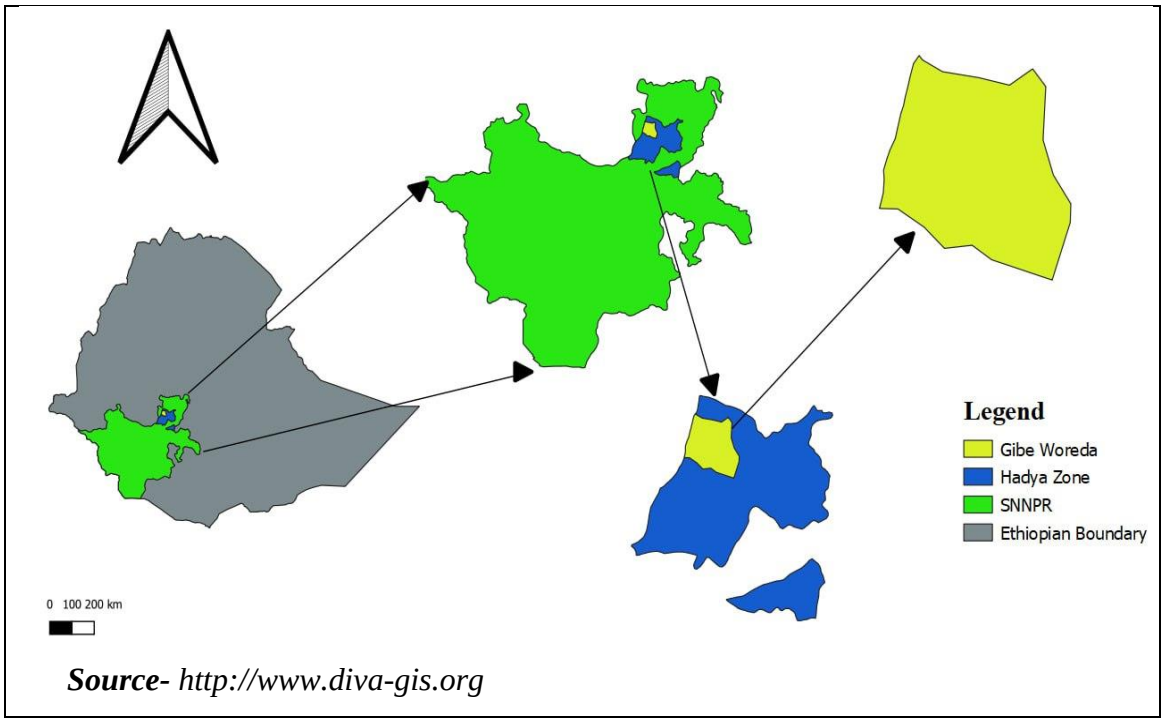


Figure 1: The map of study area.

3.2. Materials and Chemicals

Aluminum foils, Electric grinder, digital weighing balance, glove, Stereomicroscope, Magnetic stirrer, mortar and pestle, diamond pencil, Whatman No.1 filter paper, measuring cylinder, Bottom round flask, amber vials, Hot air oven, refrigerator, Petri dishes, micropipettes, Rotary evaporator with a water bath, syringe 1ml, Forceps, Incubator, spatula, Orbital shaker, and Test tubes and racks were employed in this study. In addition, Dimethyl sulfoxide (DMSO), Methanol, distilled water, commercially available acaricides; Deltamethrin (SMASH1% pour on, Tagros chemical India Pvt Ltd), Diazinon-(Vetazinon 60% EC-Adamitulu pestcides), Amitraz 12.5%-Chongqing Fangtong Animal pharmaceutical co. Ltd, different brands of Ivermectin (Tecmectin-Iver1%- Hebei yuanzheng pharmaceutical co. Itd, SG-Iver1%- Shanghai Gongyi pharmaceutical Co. Ltd, Bulvet-Iver 1%- Chongqing Bull Animal Pharmaceutical Co. LtD, JD-Iver 1%- Shijiazhuang Jiuding Animal Pharmaceutical Co. Ltd, and Ivervic-Iver 1%- Shenyang Sunvictor Pharmaceutical Co. Ltd), and phytochemical screening reagents like (Wagner's reagent, 2% and 10% NaOH, 10% lead acetate solution, concentrated. H_2SO_4, aqueous chloroform) were also used in the study.

3.3. Study Design

A cross-sectional study was conducted by questionnaire survey and experimental activity from December 2022 to April 2023. The questionnaire survey was assessed on knowledge and practice of farmers towards acaricidal use and herbal extracts against ticks in the community (Annex 1). According to the preliminary assessment, Amitraz, ivermectin, and diazinon were the most widely used commercially available acaricides in the study area and were selected for their efficacy evaluation.

3.4. Selection of Plant Material

The plants were selected based on ethnobotanical survey questionnaires that served for local farmers to specify traditional healing experiences (Annex 2) and indigenous species used to control ticks in the study area and through analysis of scientific and

ethnomedicinal information from the relevant literature. For sampling, nine peasant associations were selected purposively. To determine the sample size of respondents, the following formula was applied as described by (Ayo *et al*., 2021).

$$n = \frac{NC^2}{C^2+(N-1)e^2},$$

Where n is the sample size, *C* is the coefficient of variation (0.5), *e* is the level of precision (0.05), and *N* = 8000 households, which is the total population of the selected peasant association. Therefore, *n* = 100 respondents, which were selected randomly.

3.5. Plant Collection and Identification

The leaves of candidate plants were collected with latex gloves from their natural habitats around Gibe district in December 2022. The GPS of collection sites was recorded (Table 1; Annex 3). The harvested leaves were taxonomically identified according to (Zorloni, 2007) and authenticated by a botanist (Melaku Wondafrash) at the National Herbarium of AAU, Ethiopia, with voucher numbers (SD001, SD002, SD003, and SD004) which stand for *Calpurnia aurea*, *Datura stramonium*, *Nicotiana tobaccum*, and *Ricinus communis* respectively (Annex 4). The identified plants were transported, and deposited at Addis Ababa University College of Veterinary Medicine and Agriculture, Veterinary Pharmacology laboratory for experiment.

Table 1: The GPS records of plants collected area

Plant species	Way point (WP)	X-Easting	Y-Northing	Z (altitude in m)
C. aurea	067	0358387	0850413	2075
D. stramonium	077	0359759	0849598	2074
N. tobaccum	071	0354366	0847066	1945
R. communis	075	0359662	0849638	2079

3.6.Plant Preparation and Extraction

The gathered plant leaves were washed with distilled water to eliminate soil particles, cut into little pieces, spread out on paper sheets, dried at room temperature for two weeks,

crushed into a coarse powder, and finely powdered by an electric grinder (Annex 5). The powder was directed to extraction through the maceration by an 80% methanol (LOBA CHEMIE PVT LTD) by 1:4 (w/v) ratio of extract to solvent (Emiru *et al*., 2021). Then, dissolved and macerated for three days at room temperature (RT) (Annex 6). After 72 hours, the extract was filtered through Whatman No. 1 paper, and the final solvent was removed using a BUCHI Rota Vapour R-200 rotary vacuum evaporator at 40 ^{0}C to produce the pure crude extracts. The pure crude extracts were obtained by pouring the Rota vapour remnants into Petri dishes and storing them overnight at 40 ^{0}C in an Imperial II INCUBATOR hot air oven. Before the experiment, the prepared sections were kept at +4^{0}C (Jelalu *et al*., 2020) (Annex 7).

The extraction rate (%) was calculated as follows:

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

To prepare bioassays of extraction, the crude powder from each plant was measured and put in a separate plastic bottle. Then, 3gm of each measured crude powder was dissolved with in 30ml of 1:9 ratio of DMSO to distilled water, to prepare 100mg/ml absolute crude dilution, and shaken by a magnetic stirrer for 5 minutes and filtered using a Whatman filter paper (No.1.125 mm). The filtrate was serially diluted in to 50, 25, 12.5 and 6.25mg/ml with in 15ml of solvent. Then, transferred into labeled vials and stored at optimum temperature until the acaricidal efficacy was conducted (Annex 8).

3.7. Phytochemical Screening

Phytochemical screening was carried out at the traditional and modern drug research directorate laboratory of the Ethiopian Public Health Institute (EPHI) to assess the qualitative chemical composition and secondary metabolites of crude extract that has acaricidal activities. The standard screening tests were conducted using a conventional protocol (Annex 9) and reagents based on precipitation and color formation of

hydromethanolic extracts as described by (Sharma and Patel, 2018; Shaikh and Patil, 2020).

3.8.Tick Collection, Transportation and Identification

First, 100 animals were inspected by a thorough examination of their entire body surface to collect ticks and to estimate predominant tick species in the study area. To prevent sepsis and the possibility of an opportunistic infection, alcohol was used. From 384 ticks were collected from 25 cattle; 284(64.5%), 38(9.9%), and 100(26%) were *A. coherence*, *A. variegatum* and *R. decoloratus* respectively. Therefore, the study was designed to conduct on highly prevalent tick species in the study area. For experimental activity, ticks were collected with forceps from naturally infested cattle that had not been sprayed or injected with chemical acaricides. Finally, a total of 870 ticks (*A. coherence)* were collected and placed in perforated plastic bottles wrapped with delicate tissue (cotton) at the base for oxygen supply. Subsequently, they were transported to the Parasitology Laboratory of the Addis Ababa University College of Veterinary Medicine and Agriculture, where they were examined under a stereomicroscope for identification based on morphological characteristics like eyes, mouth part, enamel, ornamentation, leg colour, and shield colour (Walker *et al*., 2007).

3.9. Adult Immersion Test

The experiment was carried out in plant hydromethanolic leaf extracts with five different concentrations (100, 50, 25, 12.5, and 6.25 mg/ml) and three commonly used chemical acaricides (Diazinon, Amitraz) and five brands of Ivermectin (Tecmectin-Iver, SG-Iver, Bulvet-Iver, JD-Iver and Ivervic-Iver) per treatment with Deltametrin and 10% DMSO (1:9 ratio with distilled water) as positive and negative control respectively (Negero *et al*., 2014). Dilution of Diazinon and Amitraz was prepared based on the manufacturer's recommendation and guidelines on leaflets with distilled water to prepare a concentration of 0.1% and 2% (1/1000 and 1/500) respectively. In addition, deltamethrin and brands of ivermectin were diluted in to 10% (1:10) ratio in distilled water. In-vitro acaricidal

efficacy test was accompanied by using Adult Immersion Test (AIT) following Complete Randomized Design (CRD) with ten active adult ticks, counted and randomly placed in three replicate petri dishes and 5ml of each commercial acaricides and five-level of concentrations of plant extract, and 10% DMSO were directly added to the petri dishes until all ticks were immersed and left for five minutes of exposure (Heukelbach *et al*., 2006; Dinka *et al*. 2013) (Annex11).

After five minutes of contact time, the ticks were recovered from immersion by filtering with filter paper and placed in separate Petri dishes and incubated at 28°C with 80% relative humidity (Annex 11), and closely observed under a stereomicroscope at eight observation time intervals (1hr, 2hr, 4hr, 6hr, 12hr, 24hr, 48hr, and 72hr). The viability of ticks was checked by stimulation with blunt forceps and a needle and dead ticks were counted, recorded and removed (Annex 12). The percentage mortality was calculated by the formula previously used by (Krishnaveni and Venkatalakshmi, 2014) as follows.

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

3.10. Data Management and Analysis

For data management, the collected raw data were stored in a Microsoft Excel data set framework. The results concerning knowledge questions were reclassified as “correct” when the response is “yes” and “incorrect” when the answer is “no or I don’t know. The responses to questions about farmers’ practices were recorded as either “correct vs. wrong”. Data were coded by giving 1 to desirable answers and 0 to undesirable responses to a given question.

The respondents’ overall score for knowledge and practices was categorized as good, moderate, and poor for values from 80-100%, 50- 79% and <50% using a modified Bloom’s cutoff point. The overall farmers’ practices and knowledge scores would range from 0 to 7 and 0 to 5 respectively. The respondents’ overall practices and knowledge score was categorized as good, moderate, and poor if the score was ≥6 points, 4-5 points,

and <3 points for practices and ≥4 points, 3 points, and <3 points for knowledge, respectively. The final practices and knowledge scores were dichotomized for further analysis by logistic regression models, and those answers ≥50% correct (good and moderate) were considered to have sufficient (good) =1, while those answers <50% correct (score of poor) were considered to have insufficient(poor) = 0, as was previously applied by (Tufa *et al*., 2023).

The one-way ANOVA was used with Tukey's test to examine the effect of the extracts in various in vitro groups. SPSS version 26 software package computer program was used for the analysis. The mean mortality of ticks was expressed using descriptive statistics (mean ± standard error of mean, percentage, and graph). The significance level was set at $P < 0.05$. The dose-response estimation of the 50% lethal concentration (LC50) of confidence intervals of upper and lower limit was analyzed by probit analysis which was previously adapted by (Tamirat *et al*., 2014). Graphs were performed using Microsoft Excel and Graph Pad Prism 8.0.1. software, that was previously adapted by (Park *et al*., 2019).

4. RESULTS

4.1. Demographic Characteristics of the Respondents

The study showed that out of 100 respondents, 78% of them were men while 22% were women (Table 2). In that, 44% address the age group between 31-45 years, 49% for 46-60 years old, and 7% have a place with the age group over 60 years. The assessment of the educational status of the respondents indicated that 44% of them were illiterate, while 28% and 20% had completed primary and high school, respectively (Table 3) and only very few of respondents hold a College diploma 8%. Most of the farmers (94%) had >5 years of livestock farming experience.

Table 2: The demographic characteristics of respondents concerning acaricidal use

Variable	Parameters	Percent of respondent (%)
Gender	Female	22
	Male	78
Age	31-45	44
	46-60	49
	>60	7
Level of education	Illiterate	44
	Primary school	28
	High school	20
	College diploma	8
Years of livestock farming	<5 years	6
	5-10 years	22
	>10 years	72

4.2.Tick Management Practices of Farmers

All respondents complained that there was a high prevalence of tick infestation in the district and completely depended on commercial acaricides. Concerning the sources of acaricides, 59% of the farmers buy acaricides from governmental animal health clinics,

19% from veterinary pharmacies, and 22% use acaricides from open (black market). The questionnaire survey results indicated that, farmers prefer the alternative use of acaricides based on local availability and effectiveness after treatment. Accordingly, half (50%) of the interviewed farmers' favoured using ivermectin, whereas one-fourth (25%) of them preferred to use ivermectin, and less than one-fifth (14%) preferred amitraz and 11% deltamethrin (Figure 2).

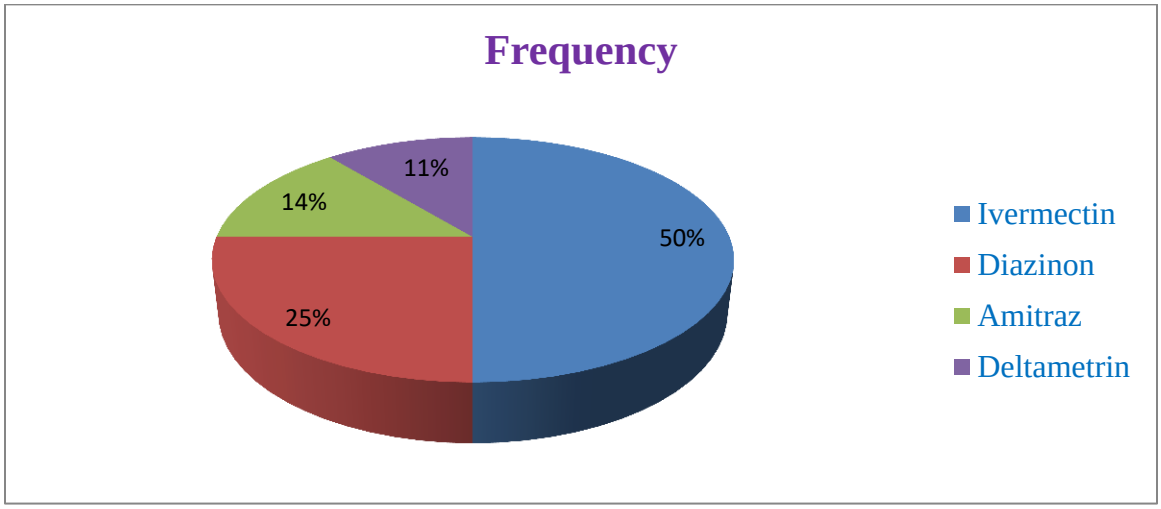


Figure 2: Commonly used acaricides in the study area

The assessment on efficacy of acaricides indicated that, half of the respondents complained that the efficacy of ivermectin, amitraz, and diazinon was very low (51%), and their effect stayed for only two months after treatment and ticks were recovered, while 49% of farmers responded as these acaricides were effective against ticks. But, the entire respondents affirmed that deltamethrin was highly effective and stood against ticks for one year. In addition, 71% of respondents asserted that these acaricides were used in the study area for more than 10 years, 25% for about 5-10 years, and 4% of respondents used them for less than five years. Concerning acaricide usage frequency, 51% of the respondents used for more than five times over the year, and 24% of the respondents used for 2-5 times, while 22% of the respondents used when needed. However, 3% of the respondents were used once in a year.

On the other hand, 29% of the farmers had awareness of use of herbals and provided one or two ethnoveterinary plants, which have acaricidal activities based on their life experience. For extraction, the herbalist used water, kerosene and urine of the cattle as the solvent. Out of these, four plants with the highest frequencies were selected for the experiment shown in (Figure 3).

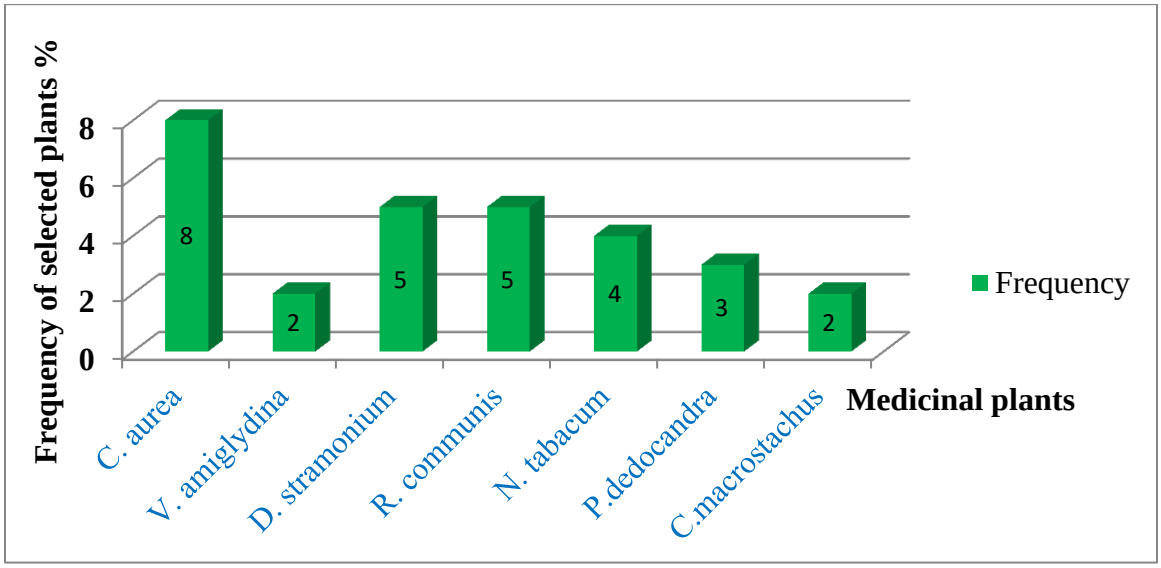


Figure 3: Responded plants for acaricidal activity in the study area.

4.3.Knowledge and Practice of Farmer's on Use of Acaricides and Herbal Extracts.

4.3.1. Knowledge

Even though, all farmers (100%) depend on acaricides to treat ticks, only 7% correctly understand what acaricides mean, and 8% of the interviewed farmers have knowledge of acaricidal residues and defined it as the presence of acaricides in milk, meat, and other animal products and how it occurs. Only 3% of the farmers know drug withdrawal periods and acaricidal resistance. Besides, 9% of the interviewed farmers have an awareness of to use/not to use dairy products until the withdrawal period of the drug. Furthermore, 84% of the respondents showed a positive attitude towards getting consultation from animal health workers and veterinarians regarding acaricidal usage, withdrawal period, and acaricidal residue before applying.

4.3.2. Practices regarding acaricidal use and resistance

The assessment showed that, 18% of the farmers had self-prescribed acaricides for their cattle, 55% of the farmers were injected and topically sprayed acaricides for their animals, and 45% called a veterinarian. In association with acaricides application on animals, 17% of the farmers observed an adverse reaction after administration. Besides, 64% of respondents used the recommended dose while 36% shared acaricides of one animal with other animals, 19% of the respondents put the reason as having no sufficient money, and 11% believed that it was sufficient, even if they use a small dose. Because of the lack of awareness, 62% of the respondents used milk for home consumption obtained from the cow under treatment conditions, while 20% gave it to calves. However, only 18% discarded it

4.3.3. Association of demographic characteristic with knowledge and practice of farmers' acaricides use

The results of the relationship between demographic characteristics with the respondent's knowledge and practice scores about acaricides use, residue, and resistance are presented in (Table 3). Univariate analysis of knowledge and practice indicated that 50% and 91% of the respondents had insufficient knowledge and practice, respectively. The two final models from a multivariate logistic analysis of the knowledge and practice of farmers showed significant results towards acaricidal use, residues, and resistance. The multivariate logistic analysis showed that age, level of education, and years of farming were strongly associated with increased levels of respondents' practice in acaricidal use, residues, and resistance. The respondents with ages 46-60 were 1.68 and 2.36 times less likely to demonstrate desirable practice and knowledge than the 31-45 age groups (p = 0.000, OR_{adj} = 0.187, CI = -2.57- 0.792) and (P = 0.029, OR_{adj} = 0.094, CI = -4.49-0.24). In addition, respondents with a college diploma were about 5.71 and 5.24 times more likely to demonstrate sufficient knowledge than illiterate and primary school (p =0.000, OR_{adj} = 0.0033, CI = -8.59-2.82) (P =0.000, OR_{adj} =0.0053, CI= -8.14-2.35), respectively. The years of farming experience had no significant association with the knowledge score.

Table 3: Multivariate logistic regression analysis on practice and knowledge of the respondents

Final generalized linear models		Coefficient	OR	95%CI	p-value
Model 1. Factors associated with sufficient practices about acaricidal use, residue and resistance					
Age	31-45	Ref			
	46-60	-1.68	.187	-2.57- 0.792	**0.000**
	>60	1.03	2.8	-1.18- 3.24	0.361
Level of education		Ref			
	Illiterate	-1.66	0.19	-2.69-0.62	**0.002**
	High school	0.66	1.94	-.601- 1.93	0.304
Years of farming	5-10 years	Ref			
	>10 years	-3.650658	0.026	-5.71-1.59	**0.001**
Model 2. Factors associated with favorable knowledge about acaricidal use					
Age	31-45	Ref			
	46-60	-2.367124	0.09375	-4.49-0.24	**0.029**
Level of education	Diploma	Ref			
	Illiterate	-5.707111	0.0033	-8.59-2.82	**0.000**
	primary school	-5.241747	0.0053	-8.14-2.35	**0.000**
Years of farming	6-10 years	Ref			
	> 10 years	-.5738002	0.56	-2.05-0 .898	0.445

CI, Confidence interval; OR adj- adjusted odd ratio; Ref- Reference of correlation; P- values in bold indicated a significant difference with the reference category (p < 0.05).

4.4. The Efficacy of Herbal Extracts Against *A. cohaerence*.

In the present study, the percentage extraction yield of the leaves of selected plants was measured and calculated after maceration with hydromethanol at (1:4) solutes to solvent ratio. The obtained crude extracts had a dark brown color, a semisolid (*D. stramonium* and *C. aurea*) and sticky consistency (*N. tobaccum* and *R. communis*) in its physical characteristics (Annex 7). The highest percentage yield was presented for the leaf of *D. stramonium* with (7.29%), followed by *N. tobaccum* and *R. communis* with yield percentages of (6.8% and 4.95%), respectively, while the lowest yield was recorded for *C. aurea*, which was observed (3.5%) as shown in (Table 4).

Table 4: The weight of herbal extracts before and after extraction

Plants	Weight of powder(g)	Weight of crude extracts(g)	Percentage of yield
C. aurea	300	10.5	3.5
D. stramonium	230	16.8	7.29
N. tobaccum	350	23.8	6.8
R. communis	400	19.8	4.95

The phytochemical screenings of the constituents were detected in the crude extracts using different reagents. The four herbal extracts were subjected to qualitative phytochemical screening tests. The result showed that all extracts have alkaloids and tannins as indicated in (Table 5; Annex 10).

Table 5: Phytochemical screening of methanolic extracts of the selected herbal extracts

Screening tests	*D. stramonium*	*C. aurea*	*N. tobaccum*	*R. communis*
Alkaloid	+	+	+	+
Flavonoid	+	+	+	-
Phenolic	+	+	+	-
Tannin	+	+	+	+
Phytosterols/ Steroid	+	+	+	-
Terpenoids	+	-	-	-
Saponin	-	+	-	+

+ = Positive, - = Negative

In vitro acaricidal efficacy of the *D. stramonium* leaf extracts against *A.coherance* was carried out in the experiment at five different concentrations level (100, 50, 25, 12.5, and 6.25mg/ml). Before 6 hours post-exposure, almost all concentrations of the extract had no activity on tick mortality. After 12hr exposure times, 100 mg/ml and 50 mg/ml concentrations of the extract resulted in (33.2±12 and 26.7±3.3%) mortality of ticks, respectively as shown in (Table 6). The results indicated a significant difference in efficacy of the five different concentrations of *D. stramonium* at 48hrs post-exposure

time when compared to 10%DMSO. While the least concentration (6.25 mg/ml) has caused significantly higher mortality when compared with 10% DMSO (negative control) at 48hr exposure time ($p = 0.036$). A significant level of ticks (63.3±6.7%) was dead at the concentration of 100mg/ml of *D. stramonium* leaf extract after 48hr post-exposure time. This showed higher efficacy when compared with amitraz and 10% DMSO ($p < 0.05$). The concentration below 50mg/ml had not shown a significant difference with in group until 72hr post-exposure time. The concentration of *D. stramonium*, 50mg/ml showed significance when compared to a concentration below 25mg/ml ($p < 0.05$). However, there was no significant mortality of ticks between concentrations 50 and 100mg/ml at 72 post-exposure times ($p > 0.05$). Finally, after 72hr post-exposure time, the one-way ANOVA showed that the mean mortality of ticks differed significantly between concentrations of *D. stramonium* and deltamethrin ($F = 56.67$, $df = 13, 28$, $p = 0.0000$). Efficacy comparisons of the means using Tukey HSD revealed a significant difference at 100mg/ml and deltamethrin (Mean= 76.7±6.7% and Mean= 100±0.00%, $p < 0.05$), respectively.

The acaricidal efficacy of the *N. tobaccum* leaf extracts of five different concentrations (100, 50, 25, 12.5, and 6.25mg/ml) against *A.coherance* were showed minimum mortality (10±5.77 and 16.7±6.7%) of ticks at 50 and 100mg/ml, respectively, with in 6hr post-exposure time ($p > 0.05$). All concentrations showed comparable mortality of ticks at 12hr post-exposure period. The concentration of 100mg/ml showed a significant mortality (46.67±8.8%) of ticks as compared to 6.25mg/ml (10±0.00%) ($p = 0.023$). A significant increase in mortality (66±8.82%) of ticks resulted from 100mg/ml at 48hr post-exposure, which was revealed a significance difference to a concentration below 25mg/ml ($p < 0.05$), while 50mg/ml caused (60±0.0%) mortality of ticks which was significant as compared to ticks immersed in a concentration of 6.25mg/ml and10% DMSO at ($F = 41.4$, $p < 0.05$). At 72hr post-exposure time, there was no significant difference of 100mg/ml compared to 50mg/ml and deltametrin, ($p > 0.05$). The maximum mortality (86.7±8.82%) of ticks was observed at 100mg/ml, while the least efficacy was recorded at 6.25gm/ml (40±5.8%) with in 72hr of post- exposure time which showed a significance difference ($F=30.2$, $p < 0.05$). Finally, 100 and 50mg/ml of *N. tobaccum* leaf

extracts showed better efficacy than amitraz and lower efficacy when compared to deltamethrin, (Figure 6).

Minimum mortality (13.3±13.3 and 6.67±6.7%) of ticks started at 6hr post-exposure time with 100 and 50mg/ml concentrations of *C. aurea*, respectively ($F= 6.76$, $p < 0.05$), relative to 10% DMSO (Table 6). After 12hr post-exposure times, 100mg/ml of the crude extract showed moderate (40±11.4%) mortality of ticks that were significant to 6.25mg/ml ($F= 7.8$, $p < 0.037$). At 24hr post-exposure time, all concentrations of the extract resulted in the comparable killing of ticks, in that 100mg/ml of the extract showed higher (56.67±12%) mortality of ticks, which was statistically significant to the concentration of 6.25mg/ml that killed (10±5.77%) of ticks ($F= 18.5$, $p < 0.05$). After 48hr post-exposure time, the three higher concentrations showed comparable levels of mortality (60±11.6, 56.7±12, and 50±11.6%) of ticks, respectively, compared to 10% DMSO at ($F= 22.4$, $p < 0.05$). The maximum mortality (63.3±8.8%) of ticks was killed at a concentration of 100mg/ml, which was significant with 6.25mg/ml that killed (33.3±13.3%) of ticks at 72 post-exposure times ($F= 33.04$, $p < 0.05$). Efficacy comparisons of the means at 72hr post-exposure using Tukey HSD did not reveal a significant difference between the extracts of *C. aurea* ($p > 0.05$), except between 100 and 6.25mg/ml ($p < 0.05$). However, all concentrations of the extract have shown lower efficacy in the mortality of ticks compared to deltamethrin (100±0.00%) at ($p < 0.05$).

A significant increase in tick mortality (20±0.0%) by 100mg/ml of the *R. communis* Leaf extracts had started at 2hr post-exposure ($F= 16.13$, $p < 0.05$) relative to 10% DMSO, concentration below 50mg/ml. All concentrations of the extracts showed weaker mortality of ticks at 4hr post-exposure time. 100mg/ml of the extract resulted in (16.7±15.3%) mortality of ticks that were statistically significant to concentration 6.25mg/ml and 10% DMSO. After 12hr up to 24hr post-exposure times, 100mg/ml resulted in significant mortality (43.3±3.3 to 63.3±6.7%) of ticks compared to 12.5mg/ml, while 50mg/ml killed (30±0.0%) of ticks that were significant to 10% DMSO ($F = 23.8$, $p < 0.05$). The maximum mortality (70±5.8%) of ticks was achieved at 100mg/ml at 48hr post-exposure time, which was significant to all concentrations below

50mg/ml, while the least efficacy (20±5.77%) was observed at 6.25mg/ml ($F = 42.9$, $p < 0.05$). The Tukey HSD test at 72hr post-exposure time revealed that deltamethrin showed higher efficacy (100±0.00%) than all concentrations of the extract ($P < 0.05$) (Table 6).

Table 6: Mean mortality (±SE) of ticks at post exposure time by different concentration of herbal extracts

Conc. (mg/ml)		Time of exposure							
		1hr	**2hr**	**4hr**	**6hr**	**12hr**	**24hr**	**48hr**	**72hr**
D. stramonium	**6.25**	.00±.00	.00±.00	.00±.00	.00±.00	6.7± 6.7	16.7±6.7	26.7 ±3.3	26.7±3.3
	12.5	.00±.00	.00±.00	.00±.00	.00±.00	16.7±8.8	20±5.8	30±5.8	40.0±5.8
	25	.00±.00	.00±.00	.00±.00	.00±.00	16.7±3.3	26.7±3.3	36.7 ±6.7	43.3±3.3
	50	.00±.00	.00±.00	.00±.00	.00±.00	26.7±3.3	40±5.8	**56.7 ±6.7**	**66.7±3.3**
	100	.00±.00	.00±.00	.00±.00	.00±.00	33.3±12	46.7±3.3	**63.3 ±6.7**	**76.7±6.7**
N. tobaccum	**6.25**	.00±.00	.00±.00	.00±.00	.00±.00	**10±0.00**	**23.3±3.3**	**33.3±3.3**	40 ±5.8
	12.5	.00±.00	.00±.00	.00±.00	.00±.00	20±5.8	30±5.8	36.7±3.3	46.7±12
	25	.00±.00	.00±.00	.00±.00	.00±.00	23.3±3.3	33.3±8.8	40±5.8	56.7±3.3
	50	.00±.00	.00±.00	.00±.00	10±5.8	33.3±6.7	46.7±3.3	**60±0.0**	66.7±3.3
	100	.00±.00	.00±.00	.00±.00	16.7±6.7	**46.7±8.8**	**56.7±3.3**	**66±8.8**	**86.7±8.8**
C. aurea	**6.25**	.00±.00	.00±.00	.00±.00	.00±.00	**.00±0.00**	**10±5.77**	**23.3 ±3.3**	**33±13.3**
	12.5	.00±.00	.00±.00	.00±.00	.00±.00	13±3.33	20±5.77	26.7±8.8	36.7±3.3
	25	.00±.00	.00±.00	.00±.00	.00±.00	20±5.77	33.3±8.8	50 ±11.6	56.7±8.8
	50	.00±.00	.00±.00	.00±.00	6.67±6.7	33.3±13.3	46.7±12	56.7 ±12	60±10
	100	.00±.00	.00±.00	.00±.00	13.3±13	**40±11.4**	**56.7±12**	**60 ±11.6**	**63.3±8.8**
R. communis	**6.25**	.00±.00	.00±.00	.00±.00	**3.3±3.3**	**10±.00**	**20±5.8**	**20±5.8**	**20±5.8**
	12.5	.00±.00	.00±.00	**3.3±5.8**	10±.00	20±5.8	26.7±3.3	33.3±3.3	40±5.8
	25	.00±.00	3.3±5.8	6.7±5.8	13.3±6.7	26.7±3.3	33.3±3.3	36.7±6.7	43.3±8.8
	50	.00±.00	6.7±5.8	10±.00	16.7±3.3	30±00	40±10	43.3±8.8	**56.7±8.8**
	100	.00±.00	**16.7±15.3**	**20±.00**	23.3±6.7	**43.3±3.3**	**63.3±6.7**	**70±5.8**	**70 ±5.8**
DMN		.00±.00	.00±.00	.00±.00	**20±5.8**	**56.7±8.8**	**90±5.8**	**100±0.0**	**100±0.0**
DMSO		.00±.00	.00±.00	.00±.00	.00±.00	.00±.00	.00±.00	.00±.0	.00±.0

The number written in bold case shown significant difference, by using ANOVA, Tukey student test (HSD), SE= Standard Error, (P < 0.05). DMN= Deltamethrin, DMSO= Dimethyl sulfoxide.

For **D. stramonium, after 72 hrs. Post exposure time, 50mg/ml is significant with 25, 12.5, 6.25mg/ml with p- value = 0.038, 0.010, 0.000 respectively and 100mg/ml is significant with 25, 12.5, 6.25mg/ml with p- value = 0.001, 0.000, 0.000 respectively. There was no significant difference between 100 and 50 mg/ml*

For **N. tobaccum, after 72 hrs. Post exposure time, 100mg/ml shown significant difference with 25, 12.5, 6.25mg/ml with p- value = 0.035, 0.001, and 0.000 respectively.*

For **C. aurea, after 72 hrs. Post exposure time, 100mg/ml shown significant difference with 6.25mg/ml with p- value = 0.035.*

For **R. communis, after 72 hrs. Post exposure time, 50mg/ml is significant with 6.25mg/ml with p- value =0.003, 100mg/ml shown significant difference with 12.5 and 6.25mg/ml with p- value = 0.028, and 0.000 respectively.*

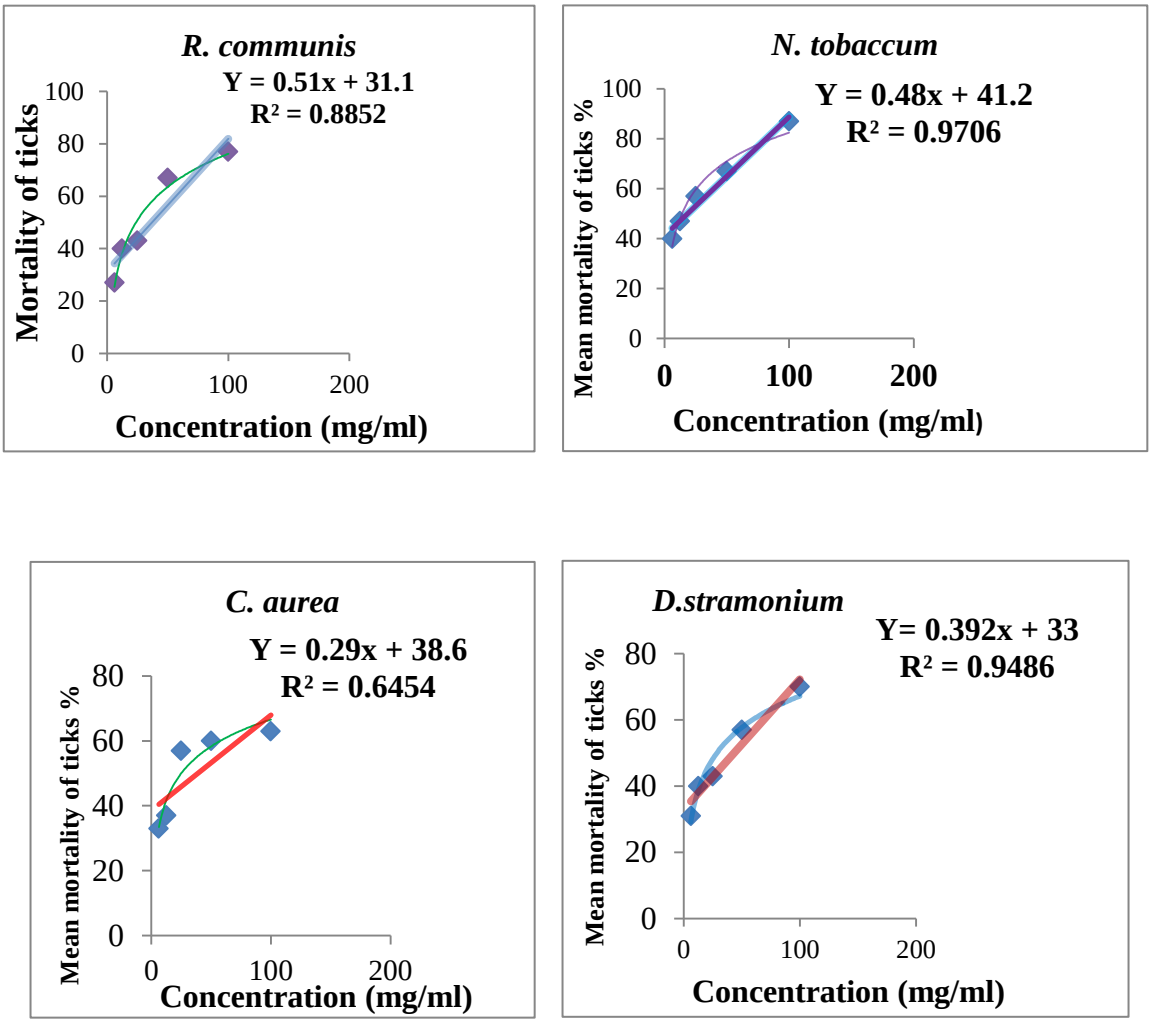
4.5. Lethal Concentration of Hydromethanolic Leaf Extracts of Selected Plants

The probit analysis of the lethal concentration of the hydromethanolic leaf extracts of selected plants was done to estimate LC50 up to LC95 at the confidence level of 95% with lower and upper confidence limits at 72hours post-exposure time, in that probit model fits well at ($P < 0.05$). Among the four herbal extracts against *A. cohaerence*, the LC50 of the *N. tobaccum* showed the highest acaricidal efficacy with a dose at a confidence interval of (9.8 - 17.9), followed by *D. stramonium* with a class limit of doses (18.7-30.3) as shown in Figure 4). Besides, *C. aurea* resulted in better acaricidal efficacy with lower and upper limits of dose (16.9-37.04), but *R. communis* showed the list efficacy record with a lower and upper limit of (20.7-40.6) compared to the other tested extracts (Table 7). Thus, *N. tobaccum* killed 50% of ticks with 13.79mg/ml, while *R. communis* killed 50% of ticks with a dose of 28.54mg/ml. According to the present study, a small dose of *N. tobaccum* killed a large population of ticks rather than other extracts.

Table 7: Probit analysis for lethal concentration of herbal extracts

Herbal extracts	Probability of death (%)	Probit (Y)	Dose	Log10 (Dose)	(LCL-UCL) of Dose	(LCL-UCL) of Log10 (Dose)
	50	5	23.82	1.377	18.7- 30.3	1.27 - 1.48
	60	5.25	39.97	1.602	31.4- 54.1	1.5- 1.73
	70	5.52	69.55	1.84	51.7- 107	1.71- 2.03
D. stramonium	80	5.84	133.00	2.12	89.7- 245	1.95- 2.39
	90	6.28	326.81	2.51	188- 792	2.28- 2.898
	95	6.64	686.66	2.84	345- 210	2.54- 3.32
	50	5	13.788	1.139	9.8-17.9	.992- 1.253
	60	5.25	24.099	1.382	18.6-31.6	1.270- 1.499
	70	5.52	43.889	1.642	33.4-63.9	1.523-1.805
N. tobaccum	80	5.84	88.421	1.947	61.2-157.5	1.787- 2.197
	90	6.28	233.57	2.368	135.8-576	2.133- 2.761
	95	6.64	520.96	2.717	258.8-1703	2.413- 3.231
	50	5	25.050	1.399	16.9-37.04	1.229- 1.569
	60	5.25	57.103	1.757	38.41-111	1.584-2.045
	70	5.52	137.89	2.140	78.7-421.1	1.896- 2.624
C. aurea	80	5.84	386.9	2.588	172.5-2116.7	2.237-3.326
	90	6.28	1618.1	3.209	498.9-20408.2	2.698-4.310
	95	6.64	5274.2	3.722	1189-133687	3.1- 5.13
	50	5	28.540	1.455	20.7- 40.6	1.315- 1.608
	60	5.25	58.173	1.765	40.9- 101.3	1.611- 2.005
R. communis	70	5.52	124.63	2.096	76.9- 296.5	1.886- 2.472
	80	5.84	303.98	2.483	155- 1083.9	2.190- 3.035
	90	6.28	1046.9	3.020	401- 6685.6	2.603- 3.825
	95	6.64	2906.6	3.463	873- 30252.8	2.941- 4.481

The probit standardized value from (5- 6.64) was taken from Finney's table for transformation of percentage of mortality to probit analysis (Finney, 1952).LCL-Lower class limit, UCL-Upper class limit



R^2= *Indicates how the values of LC50 is good to fit when it approaches to 1*

Figure 4: The regression line of the mean mortality of ticks at 72hr post exposure time.

4.6.Efficacy comparison between commercial acaricides and herbal crude extracts

Based on inferential analysis (Tukey test), all herbal extracts didn't show significant mortality of ticks by concentration below 50mg/ml. However, 100mg/ml of the extracts showed a significant difference after 48hr post-exposure. The statistical significance of one-way ANOVA on the concentration below 50mg/ml ($p > 0.05$) indicated that the efficacy of these extracts has a weak positive effect on killing ticks. At 72hr post-exposure time, *N. tobaccum* showed better efficacy (86.7±8.8%) than *D. stramonium* (76.7±6.7%). Furthermore, *R. communis* showed slightly better efficacy (70±5.8%) than

C. aurea, with a statistical mean of (63.3±8.8%). The mortality of ticks was increased with a increase in the concentration of herbal extracts as shown in (Figure 5).

In addition, deltamethrin and five brands of ivermectin (Tecmectin-Iver, SG-Iver, Bulvet-Iver, JD-Iver, and Ivervic-Iver) showed the highest (100±00%) efficacy as compared to diazinon and amitraz and concentration of all herbal extracts ($p < 0.05$), (Figure 5). Diazinon showed better efficacy (83.3±8.8%) than *D. stramonium*, *R. communis*, and *C. aurea*. All herbal extracts showed higher efficacy when compared to amitraz, with a statistical mean of (63.3±8.8%) but statistically had no significant differences ($p > 0.05$). Besides, diazinon showed a significant effect compared to a concentration below 25mg/ml, while amitraz showed significance when compared to 6.25mg/ml ($p < 0.05$). Overall, the significant value ($p < 0.05$) showed a significant difference between commercial acaricides and herbal extracts.

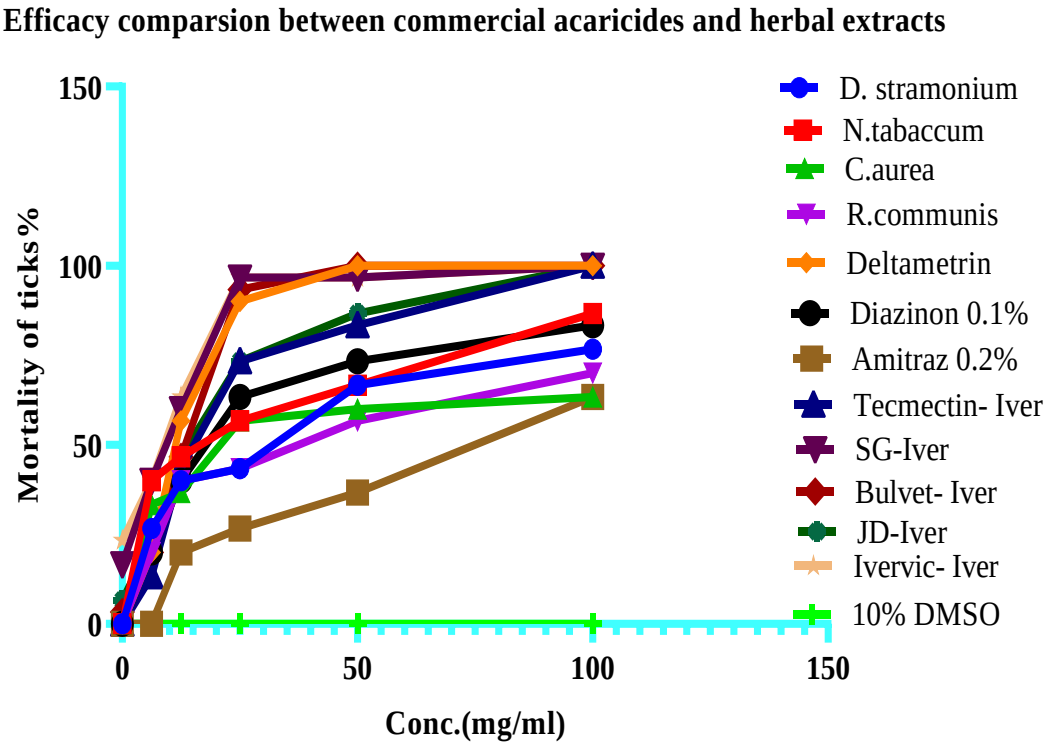


Figure 5: Efficacy comparison between herbal extracts and commercial acaricides

4.7. Statistical Analysis of the Efficacy of Acaricides

In the current study, different brands of commercial acaricides (deltamethrin, diazinon, and amitraz) with the ivermectin group (Tecmectin-Iver, SG-Iver, Bulvet-Iver, JD-Iver, and Ivervic-Iver) were applied to evaluate their efficacy by comparing with selected herbal extracts against *A. cohaerence.* Deltamethrin and 10% DMSO were used as positive and negative control, respectively, as shown in (Table 8).

Starting from 1hr post-exposure time, (14%) mortality of ticks was observed at 2hr post-exposure with Ivervic-Iver, compared to the 10% DMSO and all acaricides ($p = 0.000$). SG-Iver and Ivervic-Iver resulted in 17% and 24% mortality of ticks, respectively, at 4hr post-exposure with statistical significance, at ($p = 0.000$). At 6hr post-exposure times, JD-Iver, SG-Iver, and Ivervic- Iver showed significant mortality (23±3.3, 40±5.8, 40±5.8%) of ticks, respectively, when compared to amitraz at ($p < 0.05$). At 12hr post-exposure, all acaricides resulted in mortality of ticks. Especially, deltamethrin, SG-Iver, and Ivervic- Iver showed a significant difference in mortality of ticks, with statistical mean of (56.6±8.8, 60±5.8 and 63±3.3%) respectively, when compared to amitraz at ($p < 0.05$).

At 24hr post-exposure time, deltamethrin, diazinon, Tecmectin-Iver, Bulvet- Iver, and JD-Iver, were showed significant mortality of ticks when compared to amitraz ($p < 0.05$). Besides, SG-Iver, and Ivervic- Iver showed the higher level (96.6±3.3 and 96.6±3.3 %) of tick mortality, respectively, when compared to amitraz and diazinon at a significance level ($p < 0.05$) (Table 8). In addition, deltamethrin and Bulvet-Iver showed the highest significance over 48hr post-exposure times by killing 100% of ticks, at ($p = 0.000$) than diazinon and amitraz. Tecmectin- Iver and JD-Iver resulted in moderate efficacy by killing (83.3±6.6 and 86.7±3.3%) of ticks, respectively, at 48hr post-exposure and attained maximum (100%) mortality at 72hr post-exposure when compared to amitraz at a significance level of ($p < 0.05$). Similarly, deltamethrin and all brands of ivermectin attained maximum (100%) efficacy at 72hr post-exposure time compared to amitraz at ($p < 0.05$). Generally, the turkey test statistics of deltamethrin and brands of ivermectin were ranked highest (100%), while diazinon and amitraz were the least ranked treatment with

(83.3±8.8 and 63.3±8.8%), respectively. The mortality of ticks increased with the increase in time, as shown in (Table 8).

Table 8: Mean mortality of ticks by different brands of commercial acaricides.

Acaricides	**Mean mortality (± SE) at post exposure time**							
	1hr	**2hr**	**4hr**	**6hr**	**12hr**	**24hr**	**48hr**	**72hr**
Deltamethrin	.00±.00	.00±.00	.00±.00	20±5.8	**56.6±8.8**	**90 ±5.8**	**100 ±0.0**	**100±0.00**
Diazinon	.00±.00	.00±.00	.00±.00	20±5.8	40±10	**63.3±12**	**73.3 ±8.8**	83.3±8.8
Amitraz	.00±.00	.00±.00	.00±.00	.00±.00	20±5.8	26.7±3.3	36.6 ±3.3	63.3±8.8
Tecmectin-Iver	.00±.00	.00±.00	.00±.00	13±3.3	43±8.8	**73.3±12**	**83.3 ±6.6**	**100±0.00**
SG-Iver	.00±.00	.00±.00	**16.6±3**	**40± 5.8**	**60±5.8**	**96.6±3.3**	**96.7±3.3**	**100±0.00**
Bulvet- Iver	.00±.00	.00±.00	3.3±3	20±5.8	46.7±8.8	**93.3 ±6.6**	**100 ±0.00**	**100±0.00**
JD-Iver	.00±.00	.00±.00	6.6±3.3	**23±3.3**	46.7±3.3	**73.3±3.3**	**86.7±3.3**	**100±0.00**
Ivervic-Iver	.00±.00	**13.3±3**	**23.3±3**	**40± 5.8**	**63±3.3**	**96.6±3.3**	**96.7±3.3**	**100±0.00**
10% DMSO	.00±.00	.00±.00	.00±.00	.00±.00	.00±.00	.00±.00	.00±.00	.00±.00

 # The number written in bold case shown significant difference, by using ANOVA, Tukey student test (HSD), SE= Standard Error, (P < 0.05).

**At 2hr post exposure time, Ivervic- Iver significant with all acaricides (P = 0.000).*

**4hr post exposure time, SG- Iver significant with deltamethrin, diazinon, amitraz, Tecmectin-Iver and with p- value =0.001 and with Bulvet-Iver at p = 0.011 and Ivervic- Iver was significant with deltamethrin, diazinon, amitraz, Tecmectin-Iver, Bulvet-Iver at p- value = 0.000, and with JD-Iver at p- value = 0.001.*

**At 6hr post exposure times, SG- Iver and Ivervic-Iver were significant with amitraz and Tecmectin-Iver at p = 0.000 and 0.015 respectively, while JD-Iver was significant with amitraz, p = 0.041.*

**At 12 post exposure time, deltamethrin, SG- Iver, and Ivervic-Iver were significant with amitraz, at p = 0.029, 0.014, and 0.007 respectively.*

** At 24 and 48hr post exposure time, deltamethrin, diazinon, and all brands of ivermectin were significant to amitraz at p < 0.05. Deltamethrin and Bulvet-Iver were significant to diazinon at p = 0.008*

**At 72hr post exposure time, deltamethrin and all brands of ivermectin were significant to amitraz at p-value = 0.000*

5. DISCUSSION

The current study showed the poor knowledge and practice level of the farmers towards acaricidal use, residues, and resistance. In that, only (7%) and (8%) of the respondents clearly understood what acaricides and acaricidal residue meant, respectively. In addition, only 9% of the interviewed farmers have an awareness of not using dairy products until, the withdrawal period of the drug. Because of the lack of awareness, (62%) of the respondents were used milk from the cow under treatment conditions for home consumption. This might be a high risk for the development of drug resistance. For that matter, age, level of education, and years of livestock farming experience had a strong association (see Table 3).

The results of extracted yield in (Table 4) support the study of (Askale, 2015), who reported (9.3%) methanolic leaf extract of *R. communis*, and (Demisse and Wgebrial, 2018) reported (13.28%) ethanolic leaf extract of *D. stramonium*, respectively, but it was contrary to (Jelalu *et al*., 2020) who reported (30.6%, 22% and 20.6%) of methanolic leaf extracts of *R. communis*, *N. tobaccum*, and *C. aurea* respectively. The differences in the yields among these herbal extracts might be due to the stages of the leaves at the harvesting time, the difference in plant species, differences in altitude and latitudes where plants are found, environmental conditions, and agroecology make differences in phytochemical composition. Besides, the solvents, method of extraction, and test protocols used during extraction might cause variations in the types and concentrations of secondary bioactive compounds present in the extracts (Marie *et al*., 2023; Jelalu *et al*., 2020).

The phytochemical screening test of hydromethanolic leaf extracts of *D. stramonium*, *C. aurea*, *N. tobaccum*, and *R. communis* verified the presence of biologically active compounds in the plants (Table 5) and (Appendix 10). The leaf extracts of *D. stramonium* were positive for alkaloids, flavonoids, terpenoids, phenolic compounds, tannins, and phytosterols, while negative for saponins. This finding was in line with the study of (Richa *et al*., 2014), who reported the presence of phytosterols by using GC-MS

techniques, but did not agree with the study of (Demisse and Wgebrial, 2018), who reported positive results for alkaloids and Saponin, while negative for phenolic compound, tannins, and flavonoids for ethanolic leaf extracts of *D. stramonium*.

The study showed that, hydromethanolic leaf extracts of *C. aurea* contained phytochemicals such as alkaloids, flavonoids, phenolic compounds, tannins, phytosterols, and saponins, but not terpenoids in this study. This was similar to (Dula and Zelalem, 2018), who reported a positive result for alkaloids, saponins, phytosterols, flavonoids, phenolic compounds and tannins. Another study also showed positive for all tests, including terpenoids (Belay *et al*., 2021), which showed antioxidant and antimicrobial activity of *C. aurea* solvent fractions.

The phytochemical analysis for a crude extract of *N. tobaccum* leaf was positive for alkaloids, flavonoids, phenolic compounds, tannins, and phytosterols, while negative for terpenoids and Saponin which was aligned with the study of (Jelalu *et al*., 2020) who reported the same result of the phytochemical constitutes in *N. tobaccum* leaf extracts. But, this finding was contrary to (Oyagbemi *et al*., 2019) who reported the presence of saponins and terpenoids in addition to alkaloids, flavonoids, tannins, and anthraquinones, while found absent for steroids and cardiac glycosides. The phytochemical screening test of leaf extracts of *R. communis* was positive for alkaloid, tannin, and saponin while negative for flavonoid, phenolic compounds, phytosteroids, and terpenoids.

This result was inconsistent with the study of (Shahid *et al*., 2016), who reported the presence of all listed biologically active compounds in the seeds except flavonoids. The reason for differences in the phytochemical analysis might be the due differences in geographical location (soil type and temperature), part of plants, the season of harvesting, the type of solvent used, and the use of a highly sophisticated instrument called GC-MS for screening that can separate and identifies even smallest particles in the extract.

Having the presence of those phytochemicals in the leaf and other parts, herbal extracts provide potential activity as anti-bacterial, antifungal, anti-inflammatory, and acaricidal

agents in both human and animal health. For instance, saponin has an effect against hypercholesterolemia and regulates immune responses, steroids and terpenoids show analgesic properties, whereas alkaloids can act against chronic diseases (Divya *et al*., 2018). In addition, tannins have an effect on wound healing and inflammatory mucous membrane; alkaloids are used as antioxidant, analgesic, antimalarial, antibacterial, and anticancer agents, whereas flavonoids and phenolic act as antioxidant as well as anticancer agents, and have importance in human nutrition and health. These health benefits of flavonoids are because of their scavenging or chelating mechanism of action (Smita *et al*., 2018). The presence of those phytochemicals in the plants is a potential pointer for the development of novel drugs.

In the current study, the in vitro acaricidal efficacy evaluation of hydromethanolic leaf extracts of *D. stramonium* in (Table 6) were in line with (Srikanta *et al*., 2015), who reported (60%) mortality of adult ticks and (83%) percentage inhibition of oviposition at 100 mg/ml with ethanolic fruit extract of *D. stramonium* on *R(B). microplus* in India. In addition, these studies agree with (Demisse and Wgebrial, 2018), who report (60-80%) mortality of *B. decolrtus* with in 24hour by ethanolic crude extracts ranging from 25 to 100mg/ml and also agree with (Manzer, 2022), who found (77.17%) oviposition and (71%) mortality of larvae of *R(B). microplus* by the maximum concentration of aqueous extracts. This study was contradicted by the study of (Sisay *et al*., 2023), who reported (100%) mortality of louse by methanolic leaf extracts of *D. stramonium*. The differences in the results might be due to the type of absolute solvent, species of lice that were genetically different from ticks, presence of all phytochemicals in the crude extracts.

The results observed by different concentration of *N. tobaccum* asserted the study of (Jelalu *et al*., 2020), who reported (77%) mortality of adult *Rh. pulchellus*, by the concentration of 100 mg/ml methanolic extracts, at 24hr post-exposure times. The other study (Oyagbemi *et al*., 2019) reported (80%) mortality of *Rh. sanguineus* from dogs by N- hexane also strongly affirms these results but slightly differs from methanol extracts which resulted in (70%) mortality of ticks. The differences in this result might be due to

the low concentration of phytochemicals because of less extraction activity of methanol compared to N-hexane, which extracts all of the phytochemicals.

The results shown with *C. aurea* were in line with (Fouche *et al*., 2019), who reported (60% and 75%) of mortality of *R. turanicus* by hydroethanolic leaf and stem of the extracts, respectively, in South Africa and closely related to the study of (Tsegaye and Yosef, 2022), who reported (76.6%) mortality of ticks of goats treated with hot water extract of *C. aurea*. Likewise, (Zorloni *et al*., 2010) reported either killing or severely compromising the movement of unfed adult *Rh. pulchellus* ticks using 20% and 10% acetone leaf extracts for *C. aurea*. In contrast, these results were found to be lower than the findings of (Jelalu *et al*., 2020; Aniley and Atenafu, 2020), who reported (83.3±0.33% and 100%) mortality by methanolic and aqueous extracts of *C. aurea*, respectively. These differences might be due to genetic variation in tick species and the difference in the solvent used for extraction, in that organic solvent extracts show greater biological activity than aqueous extract (Chanda, and Parekh, 2007).

The significant increase in mortality of ticks was observed by different concentration of the hydromethanolic leaf extracts of *R. communis* which in line with (Jelalu *et al*., 2020), who reported (73.3± 0.33%) mortality by methanolic extracts of *R. communis* against *Rh. Pulchellus*. But, this study was found to be inconsistent with (Ghosh *et al*., 2013; Askale, 2015; Aniley and Atenafu, 2020), who reported (95±05%) mortality of *R(B). microplus* by hexane extract, (100%) mortality of *R(B). decoloratus* by 200 and 100 mg/ml of the methanolic extracts, and (96.7±0.54%) mortality of *A. varigatum* by 100mg/ml of aqueous extracts of leaf of the *R. communis*, respectively. These differences might be due to differences in the sensitivity of species of ticks because of genetic variation, type of solvent that extracts phytochemicals, method of extraction, and period of harvesting.

The efficacy result of deltamethrin in (Table 8) was in line with (Mehlhorn *et al*., 2011; El-Bahy *et al*., 2015), who reported (100%) mortality of nymphs and adults of *Rh. Sanguineus* in treated hair of cattle and sheep, (Thakur *et al*., 2019) reported (94.45 % and 100%) mortality at 50 ppm and beyond 50 ppm, respectively. The other study

(Kishore *et al*., 2017) also reported (100%) efficacy with 100 ppm, similar to current results. In contrast, these results were not in line with (Buczek *et al*., 2019), who reported (42.9%) of the female Ixodes ricinus ticks laid eggs at a concentration below 0.125% of deltamethrin and (Kumar *et al*.,2021) reported a (3.8 to 19.4) resistance ratio (RR50) of deltamethrin against *R. microplus*. The differences in these results might be due to the mode of application, genetically variation of tick species; continuous and indiscriminate use with improper concentrations of deltamethrin (Katuri *et al*., 2017), who reported resistance against deltametrin, cypermethrin, and diazinon.

The efficacy level of five selected brands of Ivermectin against *A. coherence* was agreed with (El-Bahy *et al*., 2015), who reported (100 %) mortality of the adult *B. annulatus* at 6hr post-exposure time. In addition, (Nava *et al*. 2019) reported high efficacy profiles of ivermectin against infestations with *R. microplus* in vivo at 7 days post-treatment, and until 21 days, the efficacy profile closed to (100%). (Diego *et al*. 2021), also reported the high efficacy of ivermectin on live animals. However, this result was higher than (Batiha *et al*., 2019), who reported (82.22% and 92.33%) mortality of *R (B). annulatus* with two brands of ivermectin at 24hr in Egypt. (Davey *et al*., 2005) found that a single dose of ivermectin 1% on cattle had an (83.2%) efficacy on adult female *R. microplus* after 12 days. Besides, (Cruz *et al*., 2015) found (89.8%) efficacy of IVM given subcutaneously under field conditions in Brazil after 14 days. Differences could be due to concentration, animal use, exposure time affecting mortality, and tick susceptibility based on species and location.

As in the case of diazinon, the current result was in line with (Eshetu *et al*., 2013), who reported (80.09±12.54%) efficacy of diazinon 0.06% EC at field recommended concentration against adult females. *A. gemma* and *R. pulchellus*, (Yilma, 2001) reported (81.05±8.65%) acaricidal effects at the highest concentration levels of diazinon on *B. decoloratus* by using AIT and (Dejene *et al*., 2021) reported (72.6% and 87.6%) efficacy of diazinon at recommended and double concentrations respectively on engorged females of *R(B).decoloratus*. But not agree with (Dinka *et al*., 2013), who reported (97.06%) mortality of engorged adult female *Rh. Pulchellus* (El-Bahy *et al*., 2015) and (Gashaw *et*

al., 2018) reported (100%) efficacy on adult *B. annulatus* and *B. decoloratus*, respectively, at 6hr post-exposure times, by using diazinon 60% at field recommended concentration. (Tamirat *et al*., 2022), reported (90% to 100%) a mortality rate at all tested concentrations by using LPT against *A. variegatum*, *B. decoloratus*, and *R. evertsi*. The difference in the result might be due to differences in preparation of concentration, method of testing, species of ticks, and duration of exposure time.

The efficacy of amitraz was (63.3±8.8%), which was comparable with (Bravo *et al*., 2008), who found (60.53%) efficacy to amitraz at concentrations of 832 ppm, which also agreed with the findings of (Mendes *et al*., 2001) and (Alliny *et al*., 2021) who reported (47.38%) mortality of ticks. Similar to the present study, (Furlong *et al*., 2007) found the mean acaricidal efficacy of amitraz as (47.9%). In Northeastern Brazil, the low acaricidal effect of amitraz (40.5% and 30.95%) was also reported by (Santana, 2000; Campos and Oliveira, 2005), respectively. In addition, (Tibebu and Assefa, 2023) was reported (57.5±0.96%) acaricidal efficacy of amitraz at Waghimra zone, northern Ethiopia. The main reason for the low efficacy of amitraz might be due to the dose recommended by the manufacturer of the product did not cause mortality to most of the ticks of this strain (Alliny *et al*., 2021). Contrary to this, most studies revealed the high acaricidal efficacy of amitraz. For instance, the study of (Dinka *et al*., 2013), who reported (90.94% and 100%) efficacy of amitraz 12.5% on oviposition inhibition and field-collected engorged adult female *Rh. Pulchellus* respectively. In addition, (Eshetu *et al*., 2013) reported a (95.47±8.7 %) mean oviposition effect of amitraz 0.025% on adult female's *A. gemma* and *R. pulchellus.* Similarly, (Gashaw *et al*., 2018) reported (98.27%) mortality of *B. decoloratus* by using amitraz 12.5% at the field recommended concentration, and the efficacy of amitraz at half recommended, recommended, and the double recommended dose was (88.9%, 98.2% and 98.86%) respectively on engorged females of *R(B).decoloratus* (Dejene *et al*., 2021). In this regard, a closely comparable finding was reported by (Sileshi Mekonnen, 2003). In South Africa, (Sileshi Mekonnen *et al*. 2002) also reported (100%) efficacy of amitraz against ticks, and (Souza *et al*. 2003) obtained (95%) mean efficacy of amitraz in Southeast Brazil.

This study has certain limitations faced during research. First, farmers don't understand what acaricides mean and their type and are confused to explain them clearly. Due to this, the quality of the data generated from the respondents has been affected by recall bias. To reduce the bias, the mode of application was used as the tool to understand the type of acaricides. Second, young age group lack awareness of herbal plants and depend on conventional drugs, which may lead to the extinction of Ethnomedicinal practice and challenging to get feedback on commonly used herbal plants in the study area. To minimize the bias, most of the respondents included in the survey were old ages. Third, restraining cattle during tick collection was energy-consuming, risky and challenging full to collect alone, which needed teamwork and high financial support for the campaign. Fourth, Seasonal variation of the study area, especially lack of rainfall affecting the proliferation of ticks, challenged in collecting sufficient number of ticks for species differentiation and know most commonly available ticks' genera in the study area. Fifth, lack of humidity and temperature maintained laboratory equipment for long-distance transportation and storage of ticks. The susceptibility of the ticks to high temperatures, cold temperatures and chemicals was challenging during collection and transportation and the zoonotic behavior of ticks and tight to the body surface. The fast movement of live ticks is a challenge to identify under the stereomicroscope.

6. CONCLUSION AND RECOMMENDATIONS

Based on this study, the knowledge and practice of farmers towards acaricidal use, residue, and resistance were very poor. However, respondents with the old age groups and college diploma level of education had moderate awareness. Both deltamethrin and all tested brands of ivermectin commonly used in the study area showed maximum efficacy against the tested genera of tick. Among alternative therapies commonly practiced by farmers in the study area, hydromethanolic leaf extracts of *D. stramonium, N. tobaccum, C. aurea,* and *R. communis* have phytochemicals with acaricidal activity against *A. cohaerence* at varied concentrations and time intervals. *N. tobaccum* showed the highest efficacy, followed by *D. stramonium, R. communis* and *C. aurea*. Compared with commercial acaricides, all herbal extracts at the concentration of 100mg/ml were showed higher efficacy than amitraz 12.5%. The mortality of ticks was increased with an increase in the concentration of herbal extracts and time of exposure. Based on the above finding, the following recommendations are forwarded.

- Veterinarians should create awareness through training to farmers on acaricidal use, withdrawal period, residues, and resistance concerning public health issues.
- Commercial acaricides that showed low efficacy should be reserved from using on animals to control tick infestation.
- In vivo toxicity and fractionation of phytochemicals should be done for extracts that showed high acaricidal efficacy.
- Extracts with higher efficacy should be used as an alternative therapy to minimize the use of commercial acaricides, thereby reduce resistance and chemical residues.

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8. ANNEXES

Annex 1: Questioner survey format

Questionnaire on practices and knowledge of livestock producers regarding chemical acaricides, resistance and herbal extracts in Gibe district of Hadiya zone, Ethiopia.

Code of respondent: _____ Address (Tell): _____

District /Town: _____ Kebele/PA: _____

Date:_____

A. Socio demographic characteristics of livestock owners/respondents

1. Which of the following best describes your gender? Female ☐ Male ☐
2. Which of the following best describes your age groups (in years)?
   ☐ ≤ 30 ☐31-45 ☐ 46-60 ☐ ≥ 61 ☐ Prefer not to answer
3. Educational level:
   ☐ Illiterate (No school /not able to read and write) ☐ Primary level (grade 1-8)
   ☐ College Diploma ☐ High school (grade 9-12) ☐ Degree (Professional)
4. Which of the following best describes your family size groups (numbers of children)? ☐ 0-3 child ☐ 4-6 child ☐ 7-10 child ☐ >10 child ☐ prefer not to answer
5. Were you raised on a livestock production? ☐ Yes ☐ No
6. If yes to Q5, which of the following best describes your number of years in livestock farming? ☐ <5 years ☐ 5-10 years ☐ >10 years ☐ prefer not to answer
7. Are they affected by tick infestation? ☐ Yes ☐ No

B. Animal Health

1. What did you do when you encountering tick infestation? More than one response is possible.
   ☐ Go to the government animal health clinic ☐ Go to a private vet clinic
   ☐ Go to a nearby veterinary pharmacy and buy medicines
   ☐ Use traditional medicines in your area
2. What kind of option did you use to treat ticks?

☐ Acaricides ☐ Herbal extracts ☐ Manual removal ☐ other (specify) _____

C. Acaricidal Use and Resistance

1. Do you know what acaricides mean? (check by e.g. in the box of drugs) *(Knowledge)*

   ☐ Yes ☐ No

2. Have you ever self-prescribed acaricides for your animals in the last month? *(Behaviour)* ☐ Yes ☐ No
3. If yes to Q2, for which animals most often do you use acaricides?

   ☐ Cattle ☐ Sheep ☐ Goats ☐ Equine ☐ others (specify) _____
4. Which acaricides are commonly used? (rank the top 5)

   (1^{st})_____, (2^{nd})_____, (3^{rd} _____, (4^{th})_____(5^{th})
5. For how long did you use them? ☐ 1-2 year ☐ 3-5 year ☐ 5-10 year ☐ 10 and above
6. How can you apply them? ☐ Injection ☐ Topical spray ☐other(specify) _____
7. Is it effective when you use them? ☐ Yes ☐ No
8. Who is administering the acaricides? *(Behavior)*

   ☐ Veterinarians (animal health professionals)

   ☐ Animal owners (non-vet)

   ☐ Other (specify)_____
9. Were any of the animals had any adverse events/reactions during administration of the acaricides (such as hives, collapsing, abortion, decrease in milk production, fever, lethargy, respiratory distress, infertility, lumps or swelling in the injection area, medicines do not work as expected, etc.)? ☐ Yes ☐ No
10. How frequently do you use acaricides over the one year?

    ☐ Once ☐ 2-5 times ☐ More than 5 times ☐ as needed
11. What are the sources of acaricides you used? (Check all that apply).

☐ Open market/ any shop ☐ Veterinary clinics ☐ Traditional practitioners

☐ Veterinary pharmacies/drug shops ☐ Community animal health worker

☐ Others specify_____

12. Do you administer the full dose and course of the acaricides as recommended? *(Behavior)* ☐ Yes ☐ No
13. If No to Q12, why not? *Multiple responses possible*

- ✓ Have no sufficient money ☐ Yes ☐ No
- ✓ Believed that it is sufficient ☐ Yes ☐ No
- ✓ Advised by others ☐ Yes ☐ No
- ✓ Others (specify) _____

14. Do you share the acaricidal of one animal with another animal? (*Behaviour*)

    ☐ Yes ☐ No

15. Do you reserve acaricidal for later use other than the current use? (*Behaviour*)

    ☐ Yes ☐ No

16. If the acaricides in your hands or those for some reason bought are expired, what do you do with them? (*Behaviour*)

    ☐ I will use them when needed ☐ Throw away

    ☐ I will not use them ☐ Return to where you bought

    ☐ Other, specify _____

17. Do you think it is important to get a consultation with a veterinarian before giving acaricides to the animals? *(Attitude)* ☐ Yes ☐ No

18. Have you heard /know about acaricidal residues? (*Knowledge*) ☐ Yes ☐ No

Are you aware of the term "drug withdrawal period"? (*Knowledge*) ☐ Yes ☐ No

19. Did the animal healthcare provider (he/she) tell you the withdrawal period of the drug used and not to use the dairy products until the end of the withdrawal period? *(Behavior)* ☐ Yes ☐ No

20. What do you do with the milk obtained from the cow under acaricidal treatment? *(Behavior)*

    ☐ Discarding ☐ Selling to the neighbour/contractors

    ☐ Giving to calves ☐ Use for home consumption

    ☐ Other, specify_____

21. Have you heard /know about acaricidal resistance (*failed to kill ticks*)? *(Knowledge)*

    ☐ Yes ☐ No

a. If Yes to Q20, would you explain it? _____

    Correct explanation ☐ Yes ☐ No

D. Use of herbal extracts against ticks

1. Dou you have awareness to use herbal extracts against ticks? *(Knowledge)*

   ☐ Yes ☐ No

2. If yes to Q1, what are the name of plants did you use locally?

   a._____ b, _____ c, _____

   d, _____e, _____ f, _____

3. By what solvents did you mix and use them?

   ☐ Water ☐ Alcohol ☐other (specify) _____

4. What is the mode of application?

   ☐ Topical spray ☐ other (specify) _____

5. Is it effective to kill ticks when you use them? ☐ Yes ☐ No

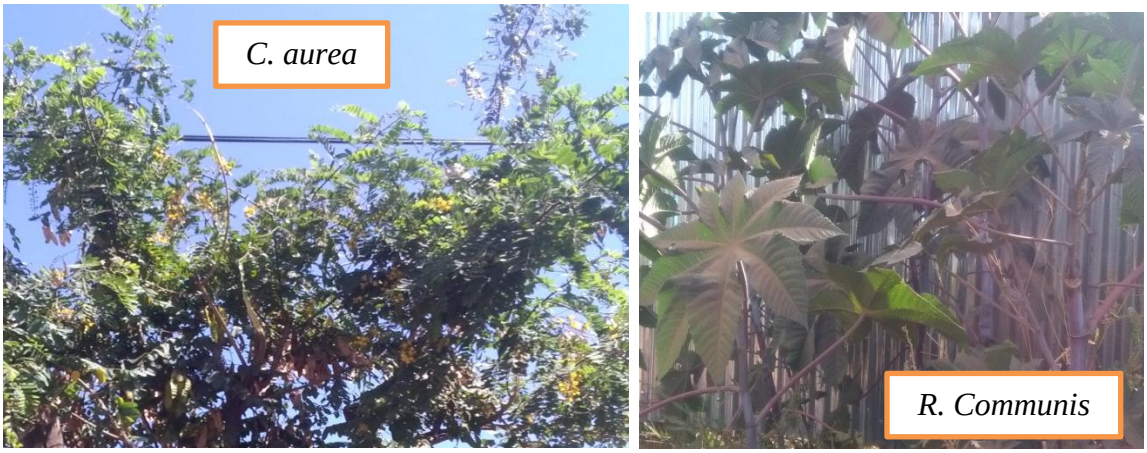
6. Do you have any additional comments about acaricidal use, resistance and containment not covered above?

   _____

Annex 2: The picture of questioner survey conducted among the farmers to get feedback about the awareness of acaricidal use and herbal extracts against ticks

Annex 3: GPS instrument used for recording the altitude and latitude of place where plants were collected.

Annex 4: The picture of collected plants for in vitro efficacy evaluation of ticks against cattle.



Annex 5: The picture of the powder of extracts

D. stramonium	*C. aurea*	*N. tobaccum*	*R. communis*

Annex 6: Crude extracts of plants under the process of maceration for 72 hours.

Annex 7: Crude extracts of plants after removal of solvent by using roter vapour.

Annex 8: Dilution of crude extracts of plants for phytochemical screening and efficacy evaluation.

Annex 9: The standard procedure for phytochemical screening test

The presence of phytochemicals in various solvent extracts of leaves of *D. stramonium, N. tobaccum, C. aurea,* and *R. communis* were analyzed qualitatively using standard tests (Sharma and Patel, 2018; Shaikh and Patil, 2020).

Alkaloid test: Wagner's test

Wagner's test: To small amount of sample Wagner's reagent (iodine in potassium iodide) was added and observed for the formation of reddish brown precipitates.

Flavonoid: Alkaline test

To 1ml of extract and 2ml of 2% NaOH solution was added and followed by the addition of few drops of diluted HCl and observed for the yellow coloration and becomes colorless on addition of diluted acid.

Phenolic compound: Lead acetate test

To small volume of sample 3ml 10% lead acetate added and observed for the bulky white precipitates.

Tannin: 10% NaOH

4ml of plant extract was dissolved in 4ml of 10% NaOH and shaken well, and observed for Formation of emulsion (Hydrolysable tannins).

Phytosterols/ steroids: Salkowski's test

Each of the extracts was treated with chloroform, and then to the chloroform layer sulphuric acid was added slowly by the sides of test tube. Formation of red color indicates the presence of steroids.

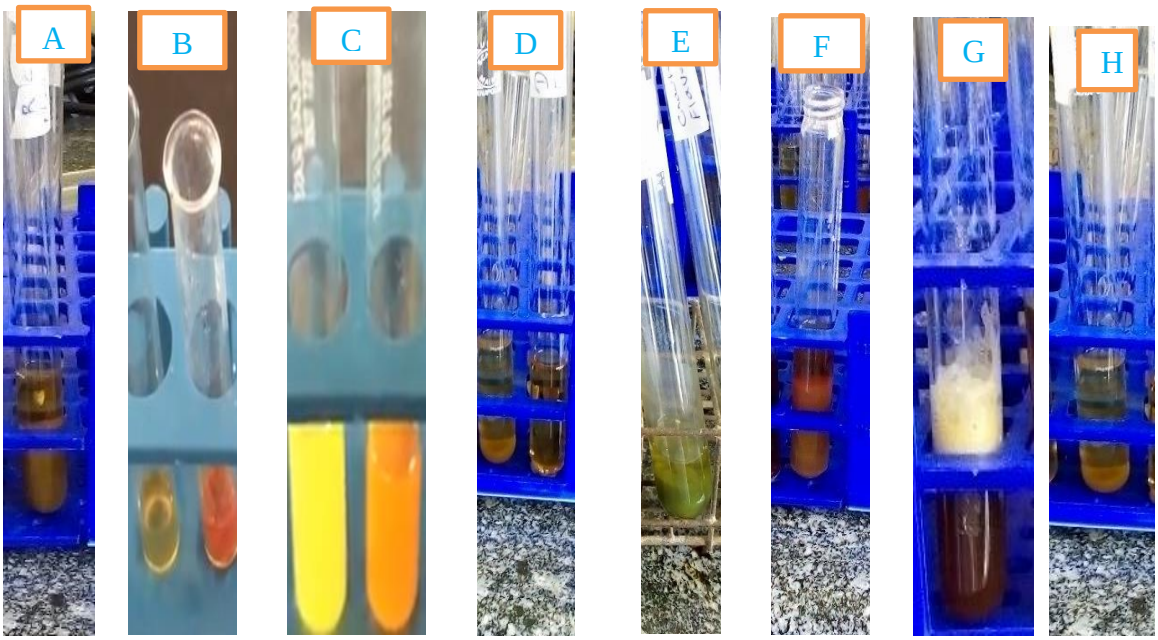
Terpenoids: Salkowski's test

To each of the extracts, 2 ml of chloroform was added. To it, 3 ml of concentrated Sulphuric acid was carefully added along the sides of the test tube to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Saponins: Frothing test

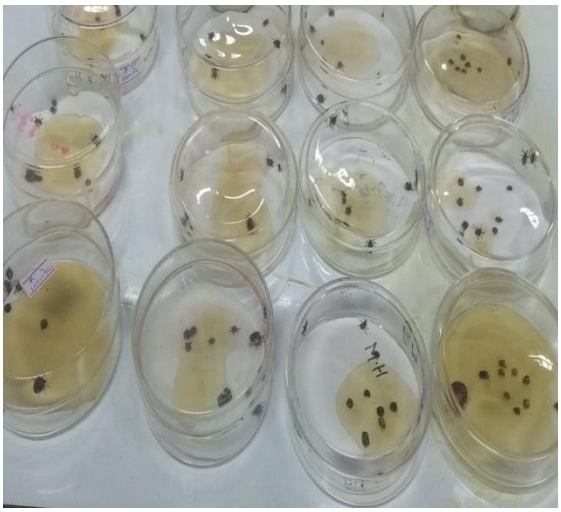
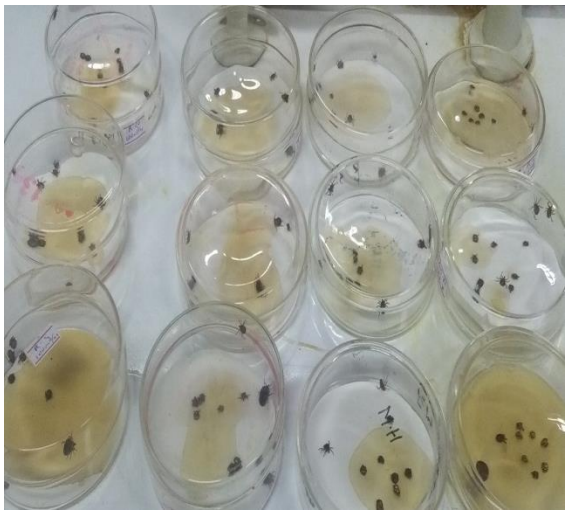
Each of the extracts was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of stable foam indicates the presence of saponins.

Annex 10: The picture of phytochemical screening test



A- Phenolic compound B- Alkaloid C- Flavonoid D- Phytosterols E- Tannin F- Terpenoids G-Saponon H-Negative control

Annex 11: The picture of ticks under efficacy test





Annex 12: The format for laboratory results recording

Plant extracts	**Conc. mg/ml**	**Time at which mortality of ticks recorded**								
		1hr	**2hr**	**3hr**	**4hr**	**6hr**	**12hr**	**24hr**	**48hr**	**72hr**
Plant-1	100									
	50									
	25									
	12.5									
	6.25									
Commercial acaricides	Dilutio 1									
	Dilution 2									

Annex 13: questioner consent form

A. Informed Consent

Assessing farmers' acaricides use and efficacy evaluation of different acaricides and selected herbal extracts against cattle ticks in Gibe district of Hadiya zone SNNPR, Ethiopia.

Consent form

Good morning/ Afternoon

I am MSc student at Addis Ababa University College of Veterinary Medicine and Agriculture and I am conducting research for my thesis on Assessing farmers' acaricides use and efficacy evaluation of different acaricides and selected herbal extracts against cattle ticks in Gibe district of Hadiya zone SNNPR, Ethiopia. The purpose of this study is to assess farmers' acaricides use, practices and efficacy evaluation of different brands of acaricides and selected herbal extracts against cattle ticks. I would like know your thoughts on acaricides use, practice, withdrawal period, resistance and residuals in animal product and use of herbal extracts. I believe that this will be very helpful in knowing the knowledge and practice of our farmers on type of chemicals they use, practice and awareness on use of animal products during treatment period of animals with treatment and endemic knowledge on use of herbal extracts against ticks of the cattle. It will produce original data that will be a tremendous help in informing future action by the public sectors and others stakeholders. In light of this it is crucial that you provide honest answers to all of the questions. Your participation in this study is entirely voluntary, and there are no personal gains or risks for you as a result. I guarantee that your response will kept and private and that any data gathered about your personal identity wont disclosed to a third party as is standard research procedure. During the report or presentation of this study, no one will learn your identity in relation to specific question and answers.

Respondents' statement: I have understood the above statement:

1. Yes (Agree to participate)
2. No (Not agree to participate)

Name of respondent: _____

Signature: _____

Date: _____