Study on ruminant tick infestation, phytochemical analysis and *in vitro* acaricidal effect of *Calpurnia aurea* and *Otostegia integrifolia* extracts on *Amblyomma variegatum*

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Abstract

Ticks limit the productivity of livestock through decreased production, reproduction, increased mortality, downgrading and rejection of hides and skin. A cross-sectional study was conducted to estimate the prevalence of tick infestation in ruminant while experimental study was used to evaluate the *in-vitro* acaricidal efficacy of methanolic extracts: *Calpurnia aurea and Otostegia integrifolia* and the phytochemicals present in those extracts at different concentrations (200, 100, 50, 25, 12.5 and 6.25 mg/ml) against *Amblyomma variegatum*. Adult immersion was used for the *in-vitro* acaricidal efficacy test and plant extracts were subjected to qualitative phytochemical screening for the presence or absence of secondary metabolites using standard procedures. Out of the 160 goats, 152 sheep and 121 cattle, 23 (14.4%), 44 (28.9%) and 28 (23.1%) were found to be positive for tick infestation, respectively. The incidence of tick infestation was significantly different ($p<0.01$) among ruminants. Five tick spp. were identified: *A. variegatum, A. gemma, R. decoloratus, R. evertsi evertsi* and *R. pulchellus*. Extract of *C. aurea* and *O. integrifolia* was found to contain alkaloids, saponins, phlobatannin, steroids, phenolic, flavonoids, glycosides and tannins. However, both plants were found negative for triterpens. Extracts of *C. aurea* and *O. integrifolia* at 200 and 100 mg/ml concentrations showed a significantly higher ($p<0.05$) acaricidal activities compared to other treatments at 24 hrs post exposure. Mortality of ticks was increased with the increased dosage (concentration) and exposure time after treatment. Extracts of *C. aurea* showed a significantly higher ($p<0.05$) tick mortality (52%) compared to those of *O. integrifolia* (27%). This is a promising finding to have alternative means of treatment and to substitute the use of synthetic drugs which have a wide spread drug resistance especially in developing countries like Ethiopia.

Key words: *Calpurnia aurea; in-vitro* test; *Otostegia integrifolia*; Phytochemical screening; Tick infestation

Introduction

Ethiopian cattle, sheep and goat population is estimated to be about 59.49, 30.70 and 30.20 million, respectively (CSA, 2017), which plays a significant role in the socio-economic life of the people. Products and by-products of livestock such as milk, meat, cheese, and butter supply the needed animal protein that contributes to the people's nutritional improvement. They also play an immense role in providing export commodities, such as meat, live animals, hides and skins to earn foreign exchange (Abunna *et al*., 2009). Even though livestock are important components of the Ethiopian farming system, their contribution to the sector are below the expected potential because they are constrained by poor feeding, poor managements and diseases (Ashenafi *et al.,* 2013). In Ethiopia, ticks are directly or indirectly involved in causing considerable financial losses to the livestock industry with an estimate of more than 1-million-birr loss per year through rejection and down-grading of hides and skins which in turn affect the tanning industries of the country (Ashenafi *et al.,* 2013).

The World Health Organization (WHO) estimated that around 80% of the population in Africa use traditional medicines and about 85% of traditional medicine involves the use of plant extracts (WHO, 2008). In Ethiopia, plant remedies are still the most important and sometimes the only sources of therapeutics for nearly 80% of human and more than 90% in livestock population. Estimated floras of 6,500 to 7,000 species of higher plants are of medically important and out of these medicinal plants 12% are endemic to Ethiopia (Giday *et al*., 2009).

Modern livestock health care is still at its immature stage in the country due to lack of adequate clinics and supply of drugs. Besides, most modern drugs are expensive and not affordable by the majority of livestock owners. As a result, people rely on their traditional knowledge, practices and locally available materials (mainly plants) in the management of diseases of their domestic animals. However, very little of the ethnoveterinary knowledge in Ethiopia in relation to the use of medicinal plants is so far properly documented and analysed (Yineger *et al*., 2008). In one hand, even though ticks are becoming the major health, productivity and breeding concerns in the farms of the Haramaya University and the surrounding district, very limited information exists or/and none at all. On the other hand, the cost of acaricides together with loss

of enzootic stability, residues in food, undesirable effects on the environment and development of resistance by ticks are some of the problems related to utilization of acaricides (De Castro, 1997).

Calpurnia aurea and *Otostegia integrifolia* were reported to be used as a control means for livestock ticks, lice and flea infestation in ethno-veterinary practice (Teklay *et al.*, 2013). In addition, the acaricidal activity of alkaloid *C. aurea* leaves extracts against *A. variegatum* was previously reported by Amante (2016). However, the *in vitro* effect of methanolic *C. aurea* and *O. integrifolia* leaves extracts against *A. variegatum* was not investigated in the current study area. In addition, there is a need for scientific based research for testing the acaricidal efficacy of these plants in order to assess alternative herbal remedies for tick and tick associated diseases. Therefore, the objectives of this study were aimed to estimate the prevalence of ruminant tick infestation and to identify ruminant tick species in and around Haramaya University farms and to evaluate the *in vitro* acaricidal activity of *C. aurea* and *O. integrifolia* methanolic extracts against *A. variegatum*.

Material and Methods

Description of the study area

Ticks were collected from Haramaya University (HU) farms and Haramaya district. The phytochemical screening test and the *in vitro* acaricidal efficacy test experiment was conducted at the laboratory of Haramaya University, College of Agriculture and Environmental Sciences. Haramaya is located in Oromia Regional State of Eastern Hararghe zone 508 km from Addis Ababa. It is located at an altitude range of 1800 to 2345 (an average 2047) m.a.s.l, 9° 26'N latitude and 42° 3'E longitude. The mean annual rainfall is 780 mm. The mean annual minimum and maximum temperatures are 8.5 and 24.4, respectively. According to the Haramaya District Rural Development and Agricultural Bureau, the district has 63,723 cattle, 13,612 sheep, 20,350 goats, 15,978 donkeys, 536 camels and 42,035 poultry.

Study animals

Study animals were ruminants (160 goats, 152 sheep and 121 cattle). Small ruminants (n=214) and cattle (n=58) were used from HU farms while small ruminants (n=98) and cattle (n=63) were used from Haramaya district vet-

erinary clinics for tick sample collection and identification. The husbandry system of dairy cattle is semi-intensive, where animals are allowed access to grazing typically in the evening. Small ruminants in the farms are kept under semi-intensive management systems which are allowed access to graze on free range land within the University both in the morning and late in the afternoon. Study population were grouped by study area, species, sex, age, breed, body condition score (BCS) and production system. Conventional age categories were made. For cattle animals aged <3 years were considered as young and ≥3 years as adult. Sheep and goats were grouped as young (2 years) and adults (≥2 years) as stated by Gatenby (2002) and as cited in Tewodros and Dawit (2015). Body condition score categorization was done according to Nicholson and Butterworth (1986).

Study design

A cross-sectional study was conducted from September 2015 to February 2017 to estimate the prevalence of tick infestation and experimental study was carried out to evaluate the *in-vitro* acaricidal efficacy test of *C. aurea and O. integrifolia* methanolic leaves extract against *A. variegatum*.

Tick collection and identification methods

Each animal was purposively sampled and subjected to physical and clinical examination and history, such as the use of acaricide treatment, concurrent disease and tick infestation associated signs including pain, lameness, and loss of appetite was recorded. Ticks were collected using forceps from the main body sites namely: head, dewlap, brisket, belly and back, udder or scrotum, anogenital, leg and tail. Adult ticks were collected and were maintained in universal bottles separately and then transported to the parasitology laboratory of College of Veterinary Medicine, HU for identification and *in vitro* acaricidal efficacy test. Date and place of collections, body sites of collection, and breed of host were recorded duly. Identification and recording of tick samples were taken place within few hours of collection. Ticks were identified using stereomicroscope following the standard identification procedures described by Walker *et al*. (2014).

Plant material collection and extraction

The leaves of plant species *C. aurea* and *O. integrifolia* were selected based on the information obtained from botanical surveys (Teklay *et al.,* 2013) in which

the community traditionally used those plants for the control of ticks. These plants were collected around from eastern Hararghe and identified and verified with taxonomical studies as reported by Zorloni (2008). The plant materials (leaves) were spread out on paper sheets in the shade at room temperature separately to dry for two weeks. The dried plant material was crushed in an electric grinder to coarse powder consistency. One hundred gram (100 gm) of powder was soaked in 200 ml of methanol separately for each plant for 48 hrs on an orbital shaker. Extracts were filtered using a Buckner funnel and Whatman (No 1 filter paper). Each filtrate was concentrated to dryness under reduced pressure at 40 °C using a rotary evaporator and the residue obtained was stored at 4°C (Eloff, 1999). The extraction rate (%) was calculated as given below:

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

Phytochemical screening of solvent extracts

The crude methanol extract was screened for the presence or absence of secondary metabolites such as alkaloids, saponins, phlobatannin, steroids, flavonoids, glycosides, phenolic compounds, tannins and triterpens using standard procedure (Tiwari *et al.,* 2011).

***In vitro* acaricidal efficacy test**

Adult immersion test

The dried extracts of C. aurea and O. integrifolia were diluted in distilled water and 3% methanol respectively, at the concentrations required for the bioassays and six concentrations were prepared arithmetically viz. 200, 100, 50, 25, 12.5 and 6.25 mg/ml by serial dilution based on FAO (2004). The in vitro tests were started within one hour after tick collection and identification. Ten active unsexed adult ticks and 3ml of each extract concentration were directly added in to each Petri-dish of the three replications. Petri-dishs were incubated at 27-28 °C and 75-80% relative humidity for 24 hrs (Sanis et al., 2012). Distilled water and 3% methanol were used as negative control for C. aurea and O. integrifolia, respectively whereas 0.1% diazinon 60 EC (Kat Relzayat Pesticides and Chemicals Co. Ltd, Egypt) was used as positive control (Jadhav et al., 2007). The test solutions, 0.1% diazinon 60 EC and distilled water were removed just

after two-minute contact time using Whatman filter paper No 1. Each tick in each Petri-dish was closely observed for death under stereomicroscope at 30 minutes, 1 hr, 2 hrs., 3 hrs., 6 hrs., 12 hrs., and 24 hrs. time intervals (Nanaa et al., 2010). The criteria used for identifying tick death were extremely strict. If any minor signs of life such as movement of head part, gut cells or minimal legs movements were observed with stimulation by forceps, the ticks were categorized as alive. The ticks were judged as dead, if there were no vital signs at all (Jadhav et al., 2007). The plant extracts were compared with ticks treated by different extract dosage, controls and different time of exposure. The numbers of fatalities were recorded in prepared format. The percent mortality rate of the ticks was calculated based on Krishnaveni and Venkatalakshmi (2014).

$$\text{Mortality \%} = \frac{\text{No. of mortality}}{\text{Total number of parasites}} \times 100$$

Accordingly, acaricidal effect was classified as strong (mortality > 80%), moderate (60-80% mortality), weak (40-60% mortality), little or no activity (mortality < 40%). Mortality in the Petri-dishes treated with extract was corrected to take account of control mortality using Abbott's correction (Pamo *et al.,* 2005).

Data entry and analysis

All data recorded in this study was entered into Microsoft excel and subsequently analyzed using STATA version 11.0 computer program. Chi-square test was used to determine the presence of association between the prevalence of tick infestation and study area, ruminant species, sex, age, breed, BCS and production system. Analysis of variance (one-way ANOVA test) was used to compare the means of tick mortality between treatments. The difference between treatments was considered significant at the ($P < 0.05$) level. Descriptive statistics was also used to review the different tick species.

Results

Identification of tick species in domestic ruminants

A significantly different ($p<0.01$) incidence of tick infestation was observed among examined domestic ruminants (cattle, sheep and goat) and Body Condition Score (Table 1). A total of 1518 ticks were collected from infested animals. Three genera and 5 species of ticks were identified. Of which, *A. variegatum*

was the dominant tick species (Table 2). The prevalence of species level tick infestation in domestic ruminants also reported (Table 3).

Table 1: Prevalence of ruminant tick infestation and associated factors

Variables	Category	Number examined	Number tick infested (%)	OR (95% CI)	p-value
Source	HU[a]	272	57 (20.9)	Reference	
	HVC[b]	161	38 (23.6)	0.6(0.31-.62)	0.520
Species	Goat	160	23 (14.4)	Reference	
	Sheep	152	44 (28.9)	2.5(0.16-1.34)	0.013
	Cattle	121	28 (23.1)	2.0(0.01-1.28)	0.046
	Total	433	95 (21.94)		
Sex	Male	60	11 (18.3)	Reference	
	Female	373	84 (22.5)	0.96(1.11-.38)	0.337
Age	Young	96	17 (17.7)	Reference	
	Adult	337	78 (23.1)	1.2(0.23-.29)	0.233
Breed	Local	325	74 (22.8)	Reference	
	Exotic	108	21 (19.4)	0.21(-.66-.54)	0.833
BCS[c]	Poor	116	47 (40.5)	Reference	
	Medium	229	38 (16.6)	4.6(-1.74-.71)	0.000
	Good	88	10 (11.4)	3.9(-2.36-.79)	0.000
Farming system	Semi intensive	272	57(21.0)	Reference	
	Extensive	161	38(23.6)	0.6(-.31-.62)	0.520

[a]Haramaya University, [b]Haramaya District Veterinary Clinic, [c]Body Condition Score

Table 2: Prevalence of tick species identified in the study areas

Tick genera	Proportion
Amblyommavariegatum	971 (64%)
Rhipicephalus (Boophilus) decoloratus	366 (24%)
Amblyommagemma	107 (7.5%)
Rhipicephalusevertsievertsi	53 (3.5%)
Rhipicephalus pulchellus	21 (1.5%)
Total	**1518 (100%)**

Table 3: Species-level tick infestation in domestic ruminants (cattle, sheep and goat)

Tick species	Cattle (n=121) (%)	Sheep (n=152) (%)	Goat (n=160) (%)	Overall prevalence
A. variegatum	14 (11.6)	20 (13.1)	12 (7.5)	46 (10.6)
Rh (B) decoloratus	6 (5.0)	13 (8.5)	8 (5.0)	27 (6.2)
A. gemma	4 (3.3)	6 (3.9)	3 (1.9)	13 (3.0)
Rh.evertsi evertsi	2 (1.7)	3 (2.0)	0	5 (1.2)
Rh. Pulchellus	2 (1.7)	2 (1.3)	0	4 (0.9)
Total	28 (23.1)	44 (28.9)	23 (14.4)	95 (21.9)

Physicochemical characteristics and yield of plant extracts

Methanolic crude extract of *C. aurea* (leaves) was green powder. The plants extract was soluble in organic solvents and fairly soluble in distilled water. The crude leaves extract of *O. integrifolia* was semi solid greenish brawn. *Otostegia integrifolia* extracts were soluble in 3% methanol (Table 4). Extract of *C. aurea* and *O. integrifolia* was found to contain different active ingredients (Table 5).

Table 4: Physical characteristics and percentage yield of *C. aurea* and *O. integrifolia* different crude extracts

Scientific names	Local name	Plant part extracted	Extraction solvent	Colour of extract	Yield (%)
C. aurea	Degeta	Leaf	Methanol	Green	17
O. integrifolia	Tunget	Leaf	Methanol	Greenish brown	19.3

Table 5: Qualitative determinations of active ingredients in crude extract of *C. aurea* and *O. integrifolia*

Scientific names	*C. aurea*	*O. integrifolia*
Alkaloids	+	-
Saponin	+	+
Phlobatannin	+	+
Steroids	+	-
Phenolic Cpds.	+	+
Flavonoids	+	-
Glycosides	+	+
Tannins	+	+
Triterpens	-	-

In vitro acaricidal activity of *C. aurea* and *O. integrifolia* against *A. variegatum*

Mortality of ticks was increased with the increased dosage (concentration) and exposure time after treatment. Extracts of *C. aurea* at 200 mg/ml and 24 hrs post exposure showed increased tick mortality (52%) compared to other exposure times (6 and 12 hrs.) within the treatments and compared to the negative control. *Calpurnia aurea* extract at 200 and 100 mg/ml concentrations showed comparable acaricidal efficacy to the reference drug (Fig 1).

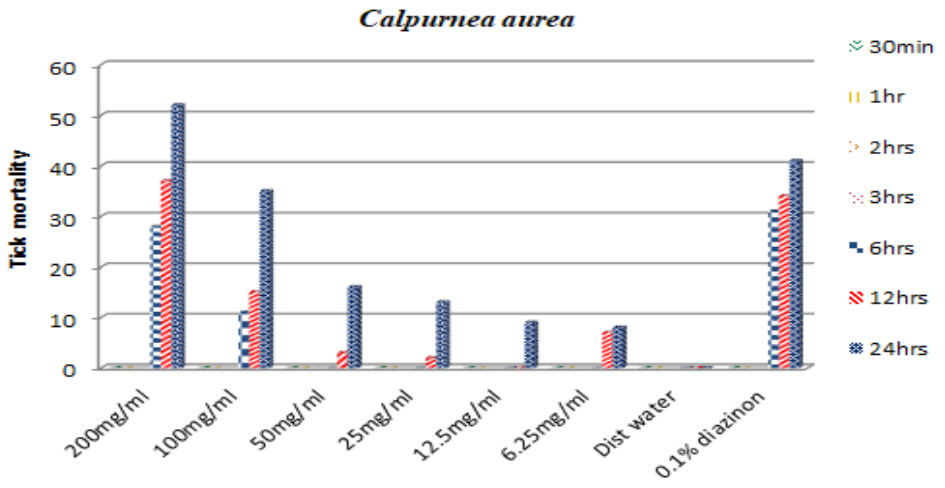

Figure 1: Mortality rate of *ticks* treated with different crude extract concentrations of *C. aurea*

Mortality of ticks was increased with the increased dosage (concentration) and exposure time after treatment. Extracts of *O. integrifolia* extract at 200 and 100 mg/ml and 24 hrs post exposure showed increased tick mortality about (26%) compared to other exposure times (6 and 12 hrs) within the treatments and compared to the negative control (Fig 2).

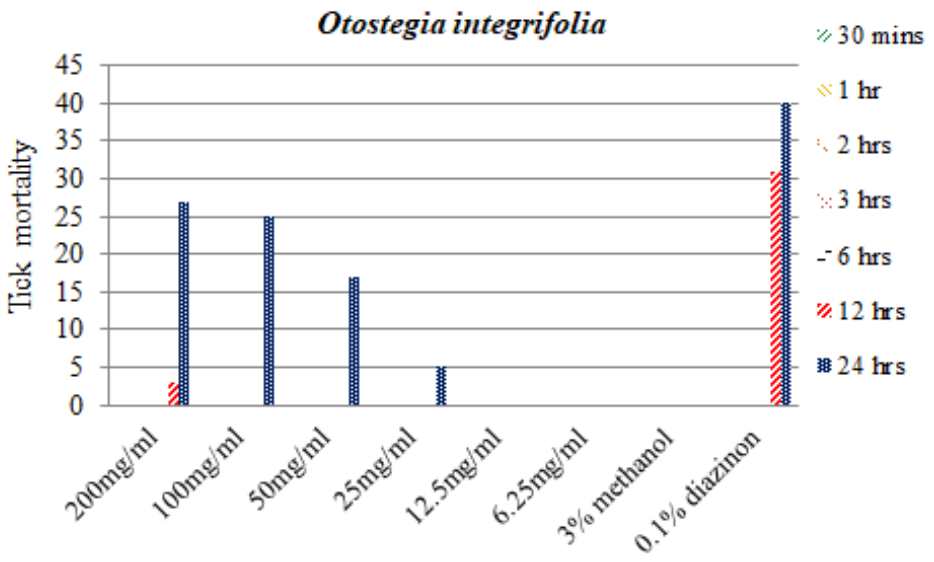

Figure 2: Mortality rate of ticks treated with different crude extract concentrations of *O. integrifolia*

The extracts of *C. aurea* at 200 mg/ml concentrations and 24 hrs after exposure showed a significantly ($p<0.05$) higher tick mortality than those of *O. integrifolia* (Table 6)

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Table 6: *In-Vitro* acaricidal efficacy evaluation of *C. aurea* and *O. integrifolia* methanolic extracts at different concentrations and time exposure against *A. variegatum*

	Mean mortality rate (%)					
	6 hrs		12 hrs		24hrs	
Dose (mg/ml)	C. aurea	O. integrifolia	C. aurea	O. integrifolia	C. aurea	O. integrifolia
200	5.3±5.3^{b}	00±00^{b}	16.3±9.5^{a}	5.5±00^{a}	51.5±5.5^{a}	26.5±5.7^{a}
100	16.3±.9.5^{a}	00±00^{b}	16.3±9.5^{a}	00±00^{b}	38.7±5.7^{b}	23±5.7^{a}
50	00±00^{c}	00±00^{b}	3.3±3.3^{d}	00±00^{b}	21.7±5.7^{c}	16±00^{b}
25	00±00^{c}	00±00^{b}	5.3±5.3^{c}	00±00^{b}	16±00^{a}	5.3±5.3^{b}
12.5	00±00^{c}	00±00^{b}	00±00^{e}	00±00^{b}	16±00^{b}	00±00^{c}
6.25	00±00^{c}	00±00^{b}	11±5.3^{b}	00±00^{b}	11±5.3^{c}	00±00^{c}
- control	00±00^{c}	00±00^{b}	00±00^{e}	00±00^{b}	00±00^{b}	00±00^{c}
+ control	5.3±5.3^{b}	6.7±3.3^{a}	11±5.3^{b}	13.3±6.7^{a}	33±3.6^{a}	38.7±5.7^{a}

Values are expressed as mean ± SD. Mean values with different letters in the same column are significantly different (P<0.05)

Discussion

In this study 21.9% of the study animals were found infested by ticks. Similar finding was also reported by Tiki and Addis (2011) who found 25.64% tick infestation around Holeta. In contrary, higher prevalence of ticks was reported from different parts of the country including 81.25% (Getachew *et al*., 2014), 74% (Meaza *et al*., 2014) and 65.5% (Wolde and Mohamed, 2014). The inconsistency among these studies could be attributed to a wide range of factors including agro-ecological, animal health practice, or managemental differences within their respective study areas.

The free-range nature of the animals most probably made significant differences of tick infestation in which cattle were two times more likely infested than goats. Whereas, animals with poor and medium body condition were more at risk for tick infestation than animals with good body condition. According to Manan *et al.* (2007), this could be due to the fact that poorly conditioned animals had low resistant to tick infestation and lack enough body capacity to build resistance whereas animals with good body condition had reasonable resistance to combat tick infestation.

Amblyomma variegatum was found to be the most abundant tick species found which accounts 64% of the total collected ticks. Similarly, prevalence data was also reported by Pawlos and Derese (2013). This finding is also in agreement with that of Ayalew *et al.* (2014) in central Oromia and Yehualashet *et al.* (1995) at Haramaya University. This was probably due to the geographic location and its being relatively active throughout the year (Hussen, 2009). Likewise, several researches, which had been conducted in different parts of Ethiopia, indicated that *A. variegatum* is the most abundant tick species with the highest prevalence. This tick species is responsible for the greatest damage to the hide and skin, because of its long mouth parts (Solomon *et al.,* 2001; Tessema and Gashaw, 2010; Tiki and Addis, 2011; Ayalew *et al.,* 2014).

The methanolic crude extract of leaves *O.integrifolia* comparatively yielded higher percentage (19.3%) than *C. aurea.* This output was supported by the report of Zewdneh *et al*. (2015) who reported similar percentage of *O. integrifolia* methanolic leaf extract. The difference on percentage yield of these extract products among the plants might be due to the difference on the nature of plant species and solvents used.

In order to know the active ingredients, present in crude extracts of the selected plants phytochemical screening test was conducted. Extract of *C. aurea* and *O. integrifolia* was found to contain alkaloids, saponins, phlobatannin, steroids, phenolic, flavonoids, glycosides and tannins. However, both plants were found negative for triterpens while *O. integrifolia*was found negative for alkaloids, steroids and flavonoids. This finding is in consistency with Umer *et al.* (2013) who reported the presence of alkaloids, tannins, flavonoids and saponins in *C. aurea* crude extract. Other study showed extract of *C. aurea* leaves was found positive for alkaloids (Zorloni *et al*., 2010).

This finding is in line with Zewdneh *et al.* (2015), who reported that methanolic extract of *O. integrifolia* (leaves) was positive for saponins and phenolic compound while negative for alkaloids, steroids and flavonoids. Parts of plant extracted and solvents used for extraction are important to determine medicinally active portions of plant (Solomon *et al*., 2013). Because the use of a different part of plant and solvent can yield in a different way in their chemical metabolites as it involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents (Ncube *et al*., 2008).

The current results revealed that the average *A. variegatum* mortality 25.5% recorded within 24 hrs of exposure of all extract concentrations of *C. aurea* at higher concentrations of 200 mg/ml and 100 mg/ml was comparable to the reference drug (positive control).This finding is different from some previous studies that reported as higher and lower average mortality of ticks. Zorloni *et al.* (2010), reported 85% of tick mortality by acetone extracts of *C. aurea* leaf at 5% concentration while Regassa (2000), found 10% of tick mortality at 5 hrs exposure of the aqueous extracts of *C. aurea* leaf and bark. The differences among these studies might be due to the difference in solvent used for extraction as studies have shown that organic solvent extracts show greater biological activity than the aqueous extract (Parekh *et al.,* 2007).

O. integrifolia showed average mortality of *A.variegatum* 12.3% at all concentrations. There is no available published scientific document on the effect of this plant against ticks. However, previous study showed the *in vivo* potent activity of hydro alcoholic leaf extract of *O. integrifolia* against *Plasmodium berghei,* malaria parasite with a maximum percent of chemo suppression of 80.5 at a dose of 600 mg/kg/day (Endale *et al*., 2013). Zorloni (2008) also reported the mosquito repellency, antimicrobial, anti-hyperglyceamic and antioxidant activities of *O. integrifolia* leaf.

Comparative *in-vitro* acaricidal activity of crude extracts of the plants revealed that *C. aurea* showed higher mortality of ticks (52%) than *O. integrifolia* (27%) after 24 hrs of exposure at 200 mg/ml concentration. The difference in mortality percentage of these plants might be due to variability in the amount of secondary metabolites among the plant extracts. The phytochemical analysis in this study showed that *C. aurea* had more secondary metabolites than *O. integrifolia.* In our study the relatively lower acaricidal activity of *O. integrifolia* is might be due to lower quantity of secondary metabolites and the absence of alkaloids, which act synergistically with glycosides when present and gave anti-tick activity (Ghosh *et al.,* 2015). Studies indicated that the presence of alkaloid, glycosides and phenol are important chemicals to initiate the mechanism of *in-vitro* and *in-vivo* action causing tick mortality (Kumar *et al.,* 2011).

Conclusion

The prevalence of tick infestation was significantly different among ruminants and body condition. Five tick species were identified: *A. variegatum, A. gemma,*

R. decoloratus, R. evertsi evertsi and R. pulchellus. *Amblyomma variegatum*. Extract of *C. aurea* and *O. integrifolia* were found to contain alkaloids, saponins, phlobatannin, steroids, phenolic, flavonoids, glycosides and tannins. Crude extracts of *C. aurea and O. integrifolia* at the higher concentrations and exposure times showed a significantly higher acaricidal activities compared to lower concentrations, exposure times and negative control. Both plants had showed comparable acarcidal effect to that of synthetic drugs (0.1% diazinon) that have been routinely used in the country. We can conclude that this is a promising finding to have alternative means of treatment to substitute the use of synthetic drugs which has a wide spread drug resistance and associated risks on the animals and human. Further detailed study on the economic losses associated with tick infestation as well as designing efficient method of tick control would have great importance. More investigation needs on their safety and efficacy *in vivo* as well as cost effectiveness of the products that exhibited considerable acaricidal activity with a view of substituting the conventional synthetic acaricide drugs. This also calls for further studies on characterization of the active ingredients of the selected plant materials. A need for further studies should be initiated to evaluate the effect of plants that showed low acaricidal effect by using a different extraction solvent and on other tick species.

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Conflict of interest

The authors declare that there is no conflict of interest.

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