**In vitro** acaricidal activity of crude extracts of *Schinus molle* (L.) leaves against field population of *Bophilus decoloratus* and *Rhipicephalus pulchellus* ticks

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Plant products are a rich source of bioactive organic chemicals and offer an advantage over synthetic pesticides as these are less toxic, less prone to development of resistance and easily biodegradable. The present study aimed at screening the acaricidal potential of crude methanolic and aqueous extracts of *Schinus molle* (L.) leaves on the adult *Bophilus decoloratus* and *Rhipicephalus pulchellus* cattle ticks, using *in vitro* immersion method. Freshly collected adult ticks were exposed to three graded concentrations of the crude extracts; 1% (1g/100ml), 2% (1g/50ml), and 4% (1g/25ml) for 24 h and mortality rates were recorded post exposure for each concentration every 3 h. Diazinon (0.2%) and distilled water were used as positive and negative controls respectively. Acaricidal activities of each concentration were measured by mean number of ticks died and antiparasitic efficacy (%) relative to the negative control. Analysis result indicated that highest (4%) and middle (2%) concentrations of both extracts caused a statistically significant (P<0.05) killing effect on *R. pulchellus* and *B. decoloratus* for most of the observation hours as compared to the extract unloaded *in vitro* groups. The relative antiparasitic efficacy (%) was highest for 4% concentration of both extracts (100%). The standard acaricide failed to completely eliminate the parasites after 24 h of exposure although it showed a slightly better effect against *B. decoloratus* (96.7%) compared to *R. pulchellus* (93.3%). Put together, this finding showed that the crude extracts of the plant have promising acaricidal properties and warrant further investigation.

**Key words:** Acaricidal, crude extracts, *Boophilus decoloratus*, *Rhipicephalus pulchellus*, *Schinus molle*.

**INTRODUCTION**

Ticks are destructive blood sucking ecto-parasites of livestock and wild animals species causing huge economic losses, thus creating food insecurity (Habeeb, 2010), with an estimated global cost of control and productivity losses of 7 billion US-Dollar annually (Nchu et al., 2012). Their effects are diverse, including reduced growth, milk production, paralysis/toxicosis, and transmission of tick-borne pathogens that reduce production or cause mortality, extensive damage to body surfaces exposing animals to secondary attacks from other
parasites and microbial infections (Walker et al., 2003). In Ethiopia, ticks and tick borne diseases cause considerable losses to the livestock economy (Bayu, 2005).

Tick control worldwide is based mainly on the repeated use of acaricides, which have resulted in problems related to environmental pollution, milk and meat contamination, and the development of resistance leading to increased cost of control (Habeeb, 2010; Pirali-Kheirabadi and Teixeira da Silva, 2011). Synthetic chemicals have also been reported to have genotoxic and cytotoxic effects on human target cells (Pirali-Kheirabadi and Razzaghi-Abyaneh, 2007). Thus, there is an urgent need for new parasitic control strategies to overcome the drawback associated with the use of synthetic drugs. One alternative control strategy could be phyto-therapy, an important component of ethno-veterinary medicine (Madzimure et al., 2011). The use of ethno-veterinary botanicals is sustainable and ecologically sound because the plants are locally available, potentially easy to be produced, locally processed and used by farmers themselves (Habeeb, 2010).

The use of botanicals for the control of ticks is compatible with traditional practices in Africa, where most resource poor-farmers use plant materials to treat endoparasites and ectoparasites of livestock (Nchu et al., 2012). Acaricidal activity of crude extracts from stem and leaves of different plants against cattle ticks has been reported (Habeeb, 2010; Rosado-Aguilar et al., 2010; Madzimure et al., 2011; Ravindran et al., 2011; Kalume et al., 2012; Vongkhamchanh et al., 2013; Dehghan-Samani et al., 2015).

_Schinus molle_ L., commonly known as pink pepper or American pepper is a tree belonging to the Anacardiaceae family which is native to subtropical regions of South America. It is a short tree with thin, long leaves often used in subtropical climates for landscaping (Taylor, 2005). _Schinus_ species has been traditionally used as medicine by indigenous people throughout the tropics (Erazo et al., 2006). In traditional cuisine, _S. molle_ fruits (berries) have been used as a replacement for black pepper and also to prepare alcoholic drinks and beverages (Marongiu et al., 2004).

In folk medicine, _S. molle_ has been used due to its antibacterial, antiviral, topical antiseptic, antifungal, antioxidant, anti-inflammatory, anti-tumoural, anti-spasmodic, analgesic properties, as well as a stimulant and an antidepressant (Alanis-Garza et al., 2007; Machado et al., 2007; Molina-Salinas et al., 2007; Guala et al., 2009). Pharmacological studies carried out with extracts from the plant validated its therapeutic properties for different ailments (Erazo et al., 2006; Machado et al., 2007; Kasimala, 2012), but there is no evidence of any effect against ticks.

In Somali Regional State of Ethiopia, _S. molle_ (Qundo berbere-Amaharic and Mirmir-Somali) is well used against ticks by pastoralists and agro-pastoralists. Based on this traditional claim, this preliminary work aimed at evaluating the _in vitro_ acaricidal activity of crude methanolic and aqueous extracts of leaves of _S. molle_ (L.) against adult _Rhipicephalus pulchellus_ and _Boophilus decoloratus_ ticks.

**MATERIALS AND METHODS**

**Study design**

This investigation employed an experimental study design; a laboratory based _in vitro_ acaricidal activity test of crude methanolic and aqueous extracts of leaves of _S. molle_ using an _in vitro_ immersion method (IIM) as described by Vongkhamchanh et al. (2013).

**Plant extracts preparation**

Fresh leaves of the plant collected from Jigjiga university botanical garden was cleaned, shade dried, mechanically ground and coarsely powdered using laboratory mortar and pestle. The powdered specimen was then subjected to extraction using two solvents; methanol and distilled water to obtain the crude methanolic and aqueous extracts respectively. The extracts were prepared by cold maceration technique. A total of 250 g of the coarsely powdered plant materials were separately soaked in each extraction solvent (100 g of powder in 1000 ml of methanol or distilled water) followed by shaking periodically for three days and then filtered. This was repeated three times to allow the solvents extract substantial quantities of the chemical constituents from the pounded plant materials. The mixture was first filtered using gauze and then the filtrate was passed through sterile filter paper (Whatman No. 3, Whatman Ltd. England). The filtered extracts were then dried in hot air oven. Finally, the resulting extracts were transferred into well labeled vials and kept in a refrigerator until required for use.

**In vitro acaricidal activity test**

The _in vitro_ acaricidal activity study was conducted using IIM on two dominant adult tick species infesting cattle in and around Jigjiga. Accordingly, unattached adult _R. pulchellus_ and _B. decoloratus_ were collected from cattle extensively reared in the vicinity of Jigjiga city, Eastern Ethiopia. All collected ticks were examined under stereomicroscope and identified to the species level using the taxonomic key described by Kaiser (2000). Adult ticks of equal size were then divided into 3 replicates of 10 each and immersed into extract concentrations of 1 (1g/100ml), 2 (1g/50ml), and 4% (1g/25ml) and incubated in petri dishes, kept at room temperature and 75% relative humidity to observe for acaricidal activity. Diazinon (0.2%) and distilled water were used as positive and negative controls respectively. The number of ticks alive or dead was counted every 3 h after exposure for 12 h and finally after 24 h. The experiment was repeated three times for precision and mean value was taken for the analysis. The antiparasitic acaricidal efficacy of each treatment was calculated using the following equation (Wang et al., 2009):

\[
AE = \frac{[B - T]}{B} \times 100\%
\]

Where AE is the antiparasitic efficacy, B is the mean number of surviving ticks in the untreated control, and T is the mean number...
Figure 1. Relative antiparasitic efficacy (%) of graded concentrations of crude methanolic and aqueous extracts of leaves of Schinus molle against adult Rhipicephalus pulchellus and Boophilus decoloratus ticks. MeOH= methanol; ext= extract.

Statistical analysis

Data were organized, edited and analyzed using statistical package for social sciences (SPSS) Version 20. Results generated from the investigation were expressed using descriptive statistics (mean ± standard error of mean, percentage, and graph). One way analysis of variance (ANOVA) was employed for analysis of differences between the in vitro groups. Results were deemed statistically significant if p≤0.05 at 95% confidence intervals.

RESULTS

The results of this study are shown in Tables 1 and 2 as mean ± SEM of dead ticks at graded concentrations of crude extracts of the plant. The analysis result indicated that both extracts of leaves of S. molle produced a relatively comparable acaricidal effects against both species of ticks when compared with the conventional acaricide, diazinon. The activity increased with concentration and time. After 3 h of exposure, only the highest concentration of the methanolic extract produced a significant tick killing effect as compared to the negative and positive controls (P<0.05). As measured by mean number of ticks died and antiparasitic efficacy (%), the methanolic extract appeared to be superior to the aqueous extract in eliminating both R. pulchellus and B. decoloratus ticks under the employed in vitro condition (Figure 1). This was particularly true at higher methanolic extract concentrations, 2 (1g/50ml) and 4% (1g/25ml).

The standard acaricide failed to completely eliminate the parasites after 24 h of exposure. It, however, showed a slightly better effect against B. decoloratus (96.7%) compared to R. pulchellus (93.3%). None of the extract unloaded ticks, that is, those exposed only to distilled water, died after 24 h of in vitro exposure.

In vitro acaricidal activity of the crude extracts against R. pulchellus

All concentrations of methanolic extract and only the highest concentration of aqueous extract elicited mortality after 3 h of exposure but progressively the ticks started dying at 6 h (Table 1). The analysis result indicated that the 4% methanolic extract (from 3 through 24 h of exposure) caused a significant acaricidal effect (P<0.05) as compared to the negative control and diazinon. Whereas, the highest (4%) and middle (2%) concentrations of aqueous extract elicited a considerable level of R. pulchellus mortality post 9 h of exposure as against the negative control (P<0.05). Mean number of surviving ticks (6.33±1.15) was highest in the in vitro groups which were exposed to 1% methanolic extract, as compared to groups loaded with other concentrations of both extracts at the end of observation period.

In vitro acaricidal activity of the crude extracts against B. decoloratus

Table 2 shows the in vitro acaricidal effect of graded...
Table 1. In vitro tick killing effect of crude leaf extracts of S. molle against R. pulchellus.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>24 MND</th>
<th>24 MNS</th>
<th>24 AE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol extract</td>
<td>1%</td>
<td>0.67±0.57</td>
<td>1.00±0.00</td>
<td>1.33±0.57</td>
<td>2.33±0.57</td>
<td>3.67±1.15&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.33±1.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.7</td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td>0.67±0.57</td>
<td>1.33±0.57</td>
<td>2.33±0.57</td>
<td>5.33±1.52&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>8.00±1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.00±1.00&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>4%</td>
<td>1.67±0.57&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>5.00±1.00&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>5.00±1.00&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>7.33±1.53&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>10.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>100</td>
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<tr>
<td>Aqueous extract</td>
<td>1%</td>
<td>0.00±0.00</td>
<td>1.67±1.15</td>
<td>3.00±1.00</td>
<td>5.00±1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.33±0.57&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.67±0.57&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>53.3</td>
</tr>
<tr>
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<td>2%</td>
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<td>2.00±1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.00±1.00&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>8.00±1.00&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1.67±1.15</td>
<td>3.67±1.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.00±1.00&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>8.33±1.15&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Diazinon</td>
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<td>0.33±0.57</td>
<td>1.00±1.00</td>
<td>2.33±0.57</td>
<td>6.00±1.00&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>9.33±0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.67±0.57&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>93.3</td>
</tr>
<tr>
<td>Distilled water</td>
<td>2ml</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>0.00±0.00</td>
<td>10.00±0.00&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>0</td>
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</table>

Values are mean ± SD; NE= Number of ticks exposed; MNS=Mean number of ticks survived; MND= Mean number of ticks died; AE= Antiparasitic Efficacy. All superscripts indicate significance at p < 0.05 (<sup>a</sup> compared to untreated; <sup>b</sup> compared to diazinon group; <sup>c</sup> compared to lowest methanolic extract concentration; <sup>d</sup> compared to lowest aqueous extract concentration).

Table 2. In vitro tick killing effect of crude leaf extracts of S. molle against B. decoloratus.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>24 MND</th>
<th>24 MNS</th>
<th>24 AE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol extract</td>
<td>1%</td>
<td>1.00±1.00</td>
<td>1.00±1.00</td>
<td>1.67±1.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.00±1.00&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>53.3</td>
</tr>
<tr>
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<td>2%</td>
<td>1.33±0.57</td>
<td>1.67±1.57&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>4.00±1.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.00±1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.67±1.52&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>13.1±1.52&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>86.7</td>
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<td>4%</td>
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<td>4.67±1.57&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>5.67±1.57&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>8.00±2.00&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>10.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>100</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>1%</td>
<td>0.00±0.00</td>
<td>0.33±0.57</td>
<td>2.33±1.52</td>
<td>3.33±1.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.67±0.57&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>56.7</td>
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<td>6.00±1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.00±1.00&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>70</td>
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<tr>
<td></td>
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<td>5.33±1.15&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>10.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>100</td>
</tr>
<tr>
<td>Diazinon</td>
<td>2ml</td>
<td>1.00±1.00</td>
<td>2.00±2.00</td>
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<td>9.00±1.00&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>9.67±0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.33±0.57&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>96.7</td>
</tr>
<tr>
<td>Distilled water</td>
<td>2ml</td>
<td>0.00±0.00</td>
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<td>0.00±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.00±0.00&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>0</td>
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</tbody>
</table>

Values are mean ± SD; NE= Number of ticks exposed; MNS=Mean number of ticks survived; MND= Mean number of ticks died; AE= Antiparasitic Efficacy. All superscripts indicate significance at p < 0.05 (<sup>a</sup> compared to untreated, <sup>b</sup> compared to diazinon group, <sup>c</sup> compared to lowest methanolic extract concentration, <sup>d</sup> compared to lowest aqueous extract concentration).

It was noted that both extracts produced a concentration dependent tickicidal effect on this species showing a strong concentration–effect relationship. Both extracts produced maximum concentrations of crude extracts of S. molle leaves against B. decoloratus.
efficacy only at their highest concentration as suggested by mean number of ticks died after 24 h of exposure (10.00±0.00).

For the *B. decoloratus*, the highest concentration of methanolic extract seemed to be superior to the highest concentration of aqueous extract at different time duration. ANOVA results indicated that highest concentration of both extracts caused a statistically significant (*P*<0.05) *B. decoloratus* killing effect for most of the observation hours as compared to the negative controls. The relative antiparasitic efficacy (%) was highest for 4% concentration of both extracts (100%) against *B. decoloratus*. However, only the methanolic extract (4%) caused 100% mortality of *R. pulchellus*. The conventional acaricide, diazinon, appeared to show slightly better efficacy against *B. decoloratus* (96.7%) compared to *R. pulchellus* (93.3%) at the end of the observation period.

**DISCUSSION**

The use of natural products, mainly acaricides from the botanical source used for the control of ticks has been the focus of research in many countries, principally to withstand the noticeable increasing frequency of acaricides resistant tick strains. In line with this trend, this preliminary work evidenced that crude methanolic and aqueous extracts of *S. molle* leaves have acaricidal effects comparable to diazinon, a conventional acaricide, justifying the traditional use of this plant against ticks. This was true especially at the highest concentration of the extracts (4%) where 100% mortality was observed after 24 h of exposure. In addition, more than 50% of tick mortality was observed as early as 12 h post exposure to the extracts. Generally, a positive correlation was noted between graded concentrations of the extracts, the exposure test-time interval and ticks mortality. This observation, in one hand, proved that crude extracts of some medicinal plants have acaricidal activities as reported by various workers (Deore and Khadabade, 2009; Magadum et al., 2009; Ribeiro et al., 2007; Ribeiro et al., 2008) and are a promising alternative for the control of ticks.

In the folk medicine, *S. molle* is an extensively studied medicinal plant throughout the world and has been reported to be used against wide ranges of human and livestock ailments (Erazo et al., 2006; Machado et al., 2007; Kasimala, 2012). In Ethiopia, its leaves are used as natural repellents against insects such as flies (Abdel-Sattar et al., 2010). To the study knowledge, there are no published studies on the acaricidal effect of *S. molle* against ticks but the results are comparable with those obtained using different medicinal plants. Most *in vitro* studies on medicinal plants often use different concentrations of extracts (varying from µgml⁻¹ to mgml⁻¹) for both adultcidal and larvicidal activities. This preliminary assay showed toxic effects against adult *B. decoloratus* and *R. pulchellus* ticks with low doses of extracts suggesting that the crude extracts have excellent potency against ticks.

The results are in line with the work of Vongkhamchanh et al. (2013), who have reported that crude extract derived from *Annona squamosa* Linnaeus leaves produced 100% adultcidal activity against cattle tick, *R. microplus* after 24 h of exposure. Borges et al. (2011) also reported *Boophilus* larval mortality rate of 100% for chloroformic extracts and 98% for hexamic extracts of *Azadirachta indica*. Similarly, Chagas et al. (2002) indicated that essential oils of *Eucalyptus citrodoro* and *Eucalyptus staigeriana* (Myrtaceae) killed 100% of the *Boophilus* tick larvae at 10% concentration. With regards to dose dependent activity, inconsonance with our observation, Ribeiro et al. (2007) also recorded that the crude extract of *Hypericum polyanthemum* produced a 100, 96.7, 84.7 and 52.7%, respectively for *Boophilus* larval mortality rates at concentrations of 50, 25, 12.5, and 6.25 mg/ml respectively after 48 h of exposure.

The extracts of the plant were more effective against *B. decoloratus* than *R. pulchellus* at different duration of time and concentration; it could be speculated that there might be variation in sensitivity to phyto-acaricides between different tick species. Furthermore, as measured by mean number of ticks died and antiparasitic efficacy (%), the methanolic extract appeared to be superior to the aqueous extract in eliminating adult ticks under the employed *in vitro* condition. This might be ascribed to the fact that difference in solvent of extraction may reveal differential activity between extracts. Plant derived compounds such as saponins, tannins, polyphenols and essential oils were reported to have acaricidal properties against ticks such as *Boophilus* (Deore and Khadabade, 2009; Magadum et al., 2009; Ribeiro et al., 2008). The anti-tick activities of the crude extracts of *S. molle* in the present study might, thus, be attributed to the presence of such biologically active acaricidal compounds. Several mechanisms have been speculated for the acaricidal activities of medicinal plants and herbal extracts: producing viscous fluids that poison and kill ticks directly; repelling ticks from individuals and populations at high risk for tick bites; attracting to the larvae as a trap to control ticks; reducing attachment of tick introduced to animals fed on diet mixed with plants; killing ticks exposed to powder or diluted extracts; reducing feeding, moulting, fecundity and viability of eggs (Abdel-Shafy et al., 2006).

**CONCLUSION**

The present study is the first report which investigated the *in vitro* acaricidal effect of the crude methanolic and aqueous extracts of *S. molle* leaves against field
population of parasitic ticks infesting cattle in and around Jigjiga, Ethiopian Somali Regional State. It was noted that both extracts of *S. molle* leaves have a promising acaricidal properties justifying the ethno-veterinary usage of the plant. This data is potentially helpful for further experimentation that encompasses larval stages, *in vivo* protocols and biological-activity-guided characterization of bio-active ingredients responsible for anti-tick activities.

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**Conflict of interests**

The authors have not declared any conflict of interests.

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