Anti-Ectoparasite Activity of Medicinal Herbal Plant in Terms of Reducing Ectoparasites Effect on Sheep and Goat Skins

by

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Abstract

Ethiopia has one of the world's largest livestock resources. However, the effects of disease, inadequate nutrition and management constrain the potential of this resource. Ectoparasites are one of the primary contributing factors in the tanneries for sheep and goat skin rejection. The aim of this study is to assess the impact of medicinal herbal plant extracts on ectoparasites (ticks) on small ruminants in Ethiopia. According to scientific and ethnomedical data gathered from respondents (farmers), the plant species P. dodecandra, E. globulus, C. macrostachyus, J. schimperiana, and C. aurea were used (by farmers) for the study. Phytochemical screening of extracts revealed the presence of flavonoids, alkaloids, phenols and saponins, tannins.

Ticks from small ruminants (i.e goat and sheep) were collected and an in vitro adult tick immersion test was carried out using concentrations of 6.25, 12.5, 25, 50, and 100 mg/ml of all medicinal plant extracts. The temporal tick mortality was observed within 24-hours. In order to compare the results, distilled water and 12.5% amitraz was used as positive and negative controls, respectively. After 24 hours of exposure, P. dodecandra, J. schimperiana, and C. macrostachyus extracts had a moderate (60%) effect on tick mortality; however, C. aurea extract at 100 mg/ml and E. globulus extract at 50 mg/ml and 100 mg/ml had the highest mortality rate (80%). The study found that following in vitro treatment for the studied plants, the mean tick mortality increased considerably with increasing concentration and exposure duration. The existence of phytochemicals (active ingredients) in several plants, such as phenols, flavonoids, alkaloids, tannin, saponin, etc., may be the cause of their anti-ectoparasite effects. The study's findings suggested that these plants might be crucial in reducing the need for chemical based medicines as well as managing the population of resistant ticks in an environmentally friendly manner.

Introduction

In Ethiopia, the agricultural sector has been prioritized by the government for encouraging overall economic growth and reducing poverty.[1] Within agriculture, the livestock subsector provides an opportunity for further growth. The Ethiopian Leather industry depends on its livestock resources, which include 54 million cattle, 25.5 million sheep, and 24 million goats, making it one of the world's largest livestock populations.[2]

The agricultural sector relies heavily on cattle, sheep, and goats as vital sources of revenue. Live animal exports as well as meat, hide, and skin are among Ethiopia's top sources of foreign exchange earnings.[1,3] Ethiopian sheep and goat skins have a good reputation for quality in the international leather market due to their fine grain and compact structure.[1,3-5]

However, due to the deterioration in skin quality brought on by an increase in ectoparasite infestations, the enormous resource potential of sheep and goat skins has been declining.[3,6-8] Parasitic skin infections brought on by ectoparasites including lice, ticks and mange are becoming a serious threat to the tanning industry.[3,5,9-11] A few years ago, tanneries in the country tended to make 70% of processed skins with grades 1-3, while 10% to 20% of the skins were considered to be of low quality.[5,10] However, over the past ten years, only 10-15% of processed skins have been deemed good, with the remainder being either rejected or downgraded as a result of an increase in ectoparasite infestations.[9,12] Consequently, Abunna

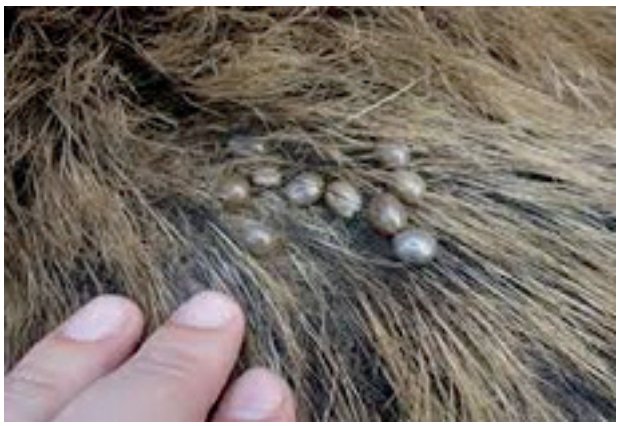

Figure 1. Tick on small ruminants

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et al.[13] reported ectoparasite prevalence in sheep (87.5%) and goat (89.9%) in Oromia region, Seyoum[11] reported prevalence in sheep (22.7%) and goat (11.5%) in Amhara region whereas Dawit et al.[14] reported prevalence in sheep (34.1%) and goat (12.2%) in Amhara region, Ethiopia.

The use of synthetic insecticides in the treatment of ectoparasites has become a serious global problem due to the emergence of ectoparasite resistance, the production of non-specific residual products, and environmental pollution. Herbal remedies are being utilized more frequently to treat a variety of ailments due to the perception that green medicine is non-hazardous, accessible, and has fewer negative impacts. The main aim of this research is to assess the effect of medicinal herbal plant extracts on ectoparasites in small ruminants in Ethiopia.

Data collection

A purposive sampling technique was carried out using group focus discussions with sheep and goat producers and field observations to collect indigenous knowledge on traditional healers. Secondary data were collected through a review of the literature published in scientific journals. Descriptive statistics were used to analyze and summarize ethno-botanical data.

Two hundred seventy (270) livestock keepers found in West Gojjam, Amhara Regional state, Ethiopia was consulted to corroborate some of the information related to ectoparasite infestation and treatment. Among these groups, semi-structured questionnaires and informal interviews were undertaken within a period of 10 days. The interviewees were shown pictures of ticks, lice and mange and requested to give the main ectoparasite species that attack their animals, the seasonal occurrence of infestation, ectoparasite species specific attachment sites and details on possible treatment methods. The information gathered shows that farmers are still relying on plant extracts as a source of ectoparasite medication for their livestock. Around 18 plants, which have medicinal value against ectoparasite were reported by farmers and that tick infestation was higher than the other ectoparasites. Cattle, followed by sheep and goats were predominantly treated by plants for worm and ectoparasite infestations. Table I indicates a list of plants used for the treatment of tick infestations in the area.

Table I

List of medicinal plants used for the treatment of ectoparasite in the area

Plant Name	Plants Parts used
Calpurnia aurea	Leaves
Phytolacca Dodecandra	Leaves
Eucalyptus globulus Labill	Leaves
Croton macrostachyus	Leaves
Azadirachta indica A. Juss	Leaves
Justicia schimperiana	Leaves
Kleinia	Leaves
Solanum incanum L.	Leaves
Aloe vera	Leaves
Acokanthera schimperi	Leaves
Clematis hirsuta	Leaves
Rumex nervosus	Leaves
Datura stramonium	Leaves
Lupinus albus	Leaves
Sida rhombifolia	Leaves
Millettia ferruginea	Leaves
Garlic	Leaves
Ginger	Leaves

Experimental

Plant Collection and drying

For the study, five (5) potential plants were chosen based on the ethnomedical information in the literature supplemented with a preliminary ethnobotanical survey during data collection from the respondents. The potential plants used for the experiment were Calpurnia *aurea*, Phytolacca *dodecandra*, Eucalyptus *globulus*, Croton *macrostachyus* and Justicia *schimperiana*. Fresh leaves of the plants were collected, shade dried for two weeks then crushed using an electric grinder into a fine powder.

Extract Preparation

Each plant powdered material was subjected to separate extraction using methanol solvent. A 100g powder was separately soaked in each extraction solvent (900 ml of solvent). As shown in Figure 2, the resulting solution was then stirred for 72 hrs. using shaker to produce the required mixtures. The mixture was filtered using Whatman No. 1 filter paper, and the extracts were kept in sealed bottles in the refrigerator (4°C) until they were required for further application.[15]

Phytochemical Analysis

Phytochemical analysis was done to determine the presence of secondary metabolites in the plants.[15]

Test for Flavonoids: A small amount of extract was treated with aqueous NaOH and HCl and observed for formation of yellow orange color.[16]

Test for Alkaloids: Mayer's reagent was used to process the extracts (1.36 g of mercuric chloride and 5 g of potassium iodide in 100 ml of water). A few ml of plant sample extract, two drops of Mayer's reagent are added along the sides of test tube. The presence of alkaloids is indicated by the formation of a yellow or white creamy precipitate.[17,63]

Test for phenols: 3 to 4 drops of 5% ferric chloride solution were added to the extracts. The presence of phenols is indicated by the formation of a bluish black or dark green color.[17,63]

Test for Tannin: In a test tube, a 2 ml portion of the extract was placed, and the extracts were treated with a few drops of 10% ferric chloride solution and observed for the formation of a blue or dark greenish color.[17,63]

Test for saponins: A 0.5 gm of extracts was mixed with 2 ml of water and shaken for 15 minutes in a graduated cylinder. The presence of saponins is shown by the formation of a 1cm layer of foam.[17.63]

Adult Immersion Test

The outcomes of an in vitro susceptibility test on the antiparasitic activity of plant-based materials are influenced by a variety of factors including plant type, method of extraction, test method and environment.[40] Ectoparasites (i.e ticks) attached to small ruminants were collected manually using thumb forceps at district level. Ticks were collected and placed in bottles covered with cotton net gauze. Within an hour of the ticks being collected, the in vitro tests were started. The effectiveness of all medicinal plant extracts as ectoparasiticides was tested in vitro (at the laboratory level) at concentrations of 100, 50, 25, and 12.5 mg/ml. In order to compare the experimental results, Amitraz 12.5% (1:1000) and distilled water were used as positive and negative controls. Five adult ticks were immersed to each extract concentration and incubated at room temperature 26° ± 2°C under a natural photoperiod. Following immersion, each tick was carefully examined for indications of death at intervals of 30 minutes, an hour, two hours, three hours, six hours, twelve hours, and twenty-four hours.[15,41] A needle was used to regularly check the condition of ticks, and if no reaction was observed, the tick was recorded as dead. Then the ecoparasiticidal efficacy of each medicinal plant extract was analyzed. The data were filled in statistical software program (SPSS) and descriptive statistics were used to summarize the ethno-botanical data in terms of tables and graphs.

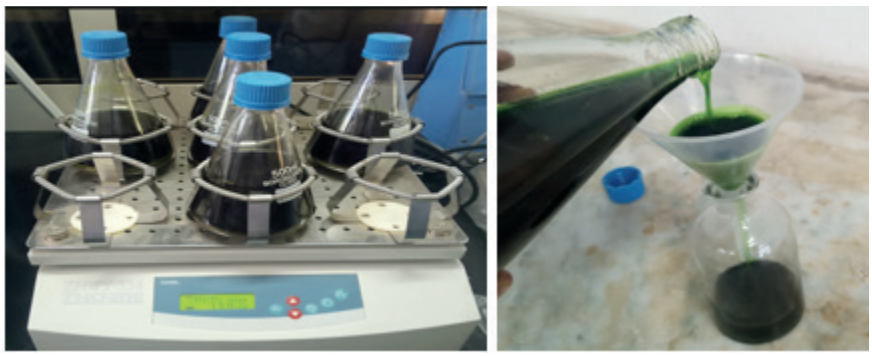

Figure 2. Extract shaking and filtration

Table II
Phytochemical analysis

Compound	Test Method	Methanolic extract				
		C. *aurea*	P. *dodecandra*	E. *globulus*	J. *schimperiana*	C. *macrostachyus*
Flavonoids	Alkaline reagent[16]	+	+	++	++	++
Alkaloids	Wagner's test[17]	+	+	+	++	++
Phenols	Ferric chloride[17]	+	++	-	++	+
Tannins	Ferric chloride[17]	+	++	++	++	++
Saponins	Frothing[17]	+	+	++	+	+

Key: (+): Weak positive test and (++), Strong positive test, (-) Not detected

Result and Discussion

Preliminary Qualitative Analysis

As shown in Table II, phytochemical screening of methanolic extracts have significant indication about the presence of secondary plant metabolites such as, phenolic compounds, alkaloids, flavonoids, tannins, saponin, and steroids.

Flavonoids Detection: The phytochemical analysis of methanolic extracts using the alkaline reagent test revealed a strong positive (++) presence of flavonoids in E. *globulus*, J. *schimperiana*, C. *macrostachyus* and a weak positive (+) presence of flavonoids in C. *aurea* extracts as shown in Table II. Correspondingly, different studies revealed the presence of flavonoids in the extract of C. *aurea,*[18] E. *globulus,*[19] J. *schimperiana*[20] and C. *macrostachyus* extracts.[21]

Alkaloids detection: According to Table II, the Mayer's and Wagner's tests showed that the extracts of J. *schimperiana* and C. *macrostachyus* had strong positive (++) alkaloids, whereas the extracts of C. *aurea*, P. *dodecandra*, and E. *globulus* had weak positive (+) alkaloids. Correspondingly, different studies revealed the presence of alkaloids in the extract of C. *aurea,*[18] P. *dodecandra*,[22] E. *globulus,*[23] J. *schimperiana*[24] and C. *macrostachyus.*[25]

Phenols detection: According to Table II, the ferric chloride test indicated that the extracts of P. *dodecandra* and J. *schimperiana* had strong positive (++) phenols, while the extracts of C. *aurea* and C. *macrostachyus* contained weak positive (+) flavonoids. Accordingly, several studies have demonstrated the presence of phenols in the extracts of C. *aurea,*[26] P. *dodecandra,*[22] J. *schimperiana*[24] and C. *macrostachyus.*[27]

Tannin detection: According to the results of the ferric chloride test, strongly positive (++) tannin were detected in the extracts of P. *dodecandra*, E. *globulus*, J. *schimperiana*, and C. *macrostachyus* and weakly positive (++) tannins were detected in C. *aurea*, which were shown in Table II. Consequently, various studies have demonstrated the presence of tannin in the extracts of C. *aurea,*[28] P. *dodecandra,*[29] E. *globulus,*[30] J. *schimperiana*[31] and C. *macrostachyus.*[21]

Saponins Detection:

Following Froth test approach, the extracts of E. *globulus* showed a strong positive (++) saponins, while the extracts of C. *aurea*, P. *dodecandra*, J. *schimperiana*, and C. *macrostachyus* indicated the presence of weak positive (+) flavonoids, which were indicated in Table II. Consequently, several studies have confirmed the existence of saponins in the extracts of C. *aurea,*[18] P. *dodecandra,*[32] E. *globulus,*[33] J *schimperiana*[20] and Croton *macrostachyus.*[27]

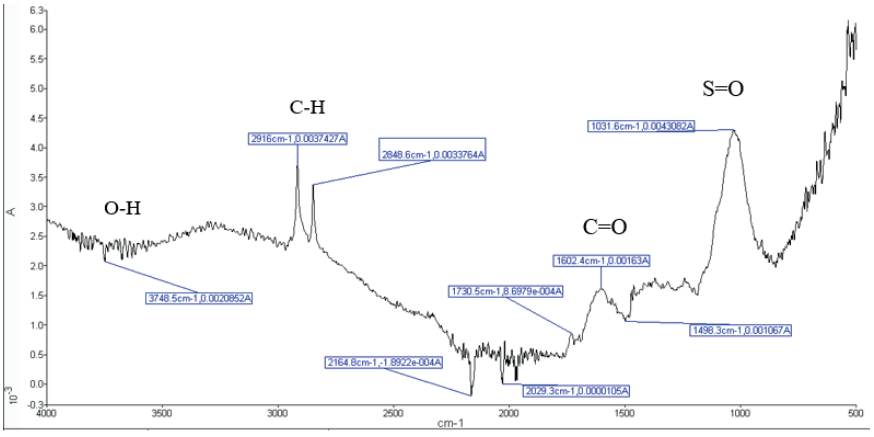

Figure 3. FT-IR absorbance spectrum of Calpurnia aurea

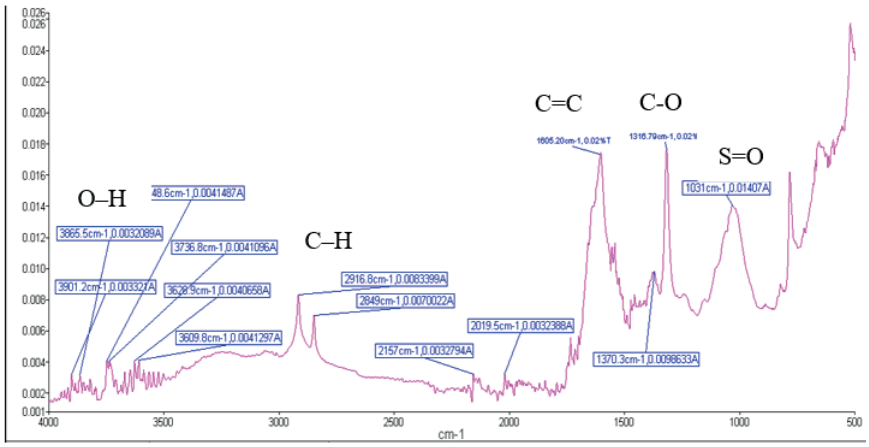

Figure 4. FT-IR absorbance spectrum of Phytolacca Dodecandra

FTIR Analysis

Fourier Transform Infrared Spectroscopy (FTIR) was used to identify different characteristics and functional groups that were present in various materials.[34] The FTIR data spectrum was recorded with a scan speed of 2 mm/s and the powdered plant samples were added as a powder with a wavelength spectral range of 4000cm^{-1}-500 cm^{-1}.[35]

The above Figure 3 shows the functional groups present in the C. *aurea* leaves. In the FT-IR spectrum of C. *aurea*, the region from 2950cm^{-1}-2800cm^{-1} indicate the presence of stretching vibration of C-H of alkane functional groups.[36] The presence of carboxylic acid (O–H stretch) was revealed by the peak 3750 cm^{-1}. The region from 1700cm^{-1}-1500cm^{-1} indicates the presence of asymmetric carboxyl stretching vibration of C=O functional groups.[37] A strong absorption broad peak at 1030^{cm-1} indicate the presence S=O stretching due to sulfoxide functional groups.[38]

As shown in Figure 4, the FT-IR spectrum of Phytolacca D. revealed the presence of carboxylic acid (O–H stretch) at a weak peak at 3750 cm–1 and the medium peak appeared in the range of 2900–2850 cm–1 mainly attributed to the stretching vibration of C–H of alkane. The medium peak appeared at 1606 cm–1 and 1310 cm–1 mainly attributed to C=C stretching vibration and C-O stretching vibration, respectively. The medium stronger peak appears at 1034 cm^{-1} attributed to S=O stretching due to sulfoxide functional groups.

As shown in Figure 5, the FT-IR spectrum of Eucalyptus *globulus* provided a weak peak at 3748 cm^{-1} which indicated the presence of O-H stretching due to alcohols.[34] It showed peaks at about 1622cm^{-1} attributed to C=C stretching due to alkenes. The broad peak at 1034 cm^{-1} attributed to S=O stretching due to sulfoxide functional groups of compound. FT-IR spectrum confirmed the presence of secondary plant metabolites, which are alcohols, phenols, alkenes and sulfoxides in plant extracts.[39]

As shown in Figure 6, the IR spectrum of Justicia *schimperiana* peaks near 3748 cm-1 showed absorption bands assigned to an O-H stretching of alcohol. Two sharp peaks near to 2917 cm-1 and 2849 cm-1 are indicative of C-H stretching of Alkane. It also revealed the presence of a weak anhydride CO-O-CO stretching at 1040 cm-1. Therefore, the IR spectrum depicts the presence of alcohol, alkane and anhydride groups attached to a quaternary carbon in the compound.

As shown in Figure 7, the FT-IR spectrum of Croton *macrostachyus*, the absorption band at 3745 cm-1 indicated the presence of O-H stretching of alcohol whereas the absorption bands at 2917 cm-1 and

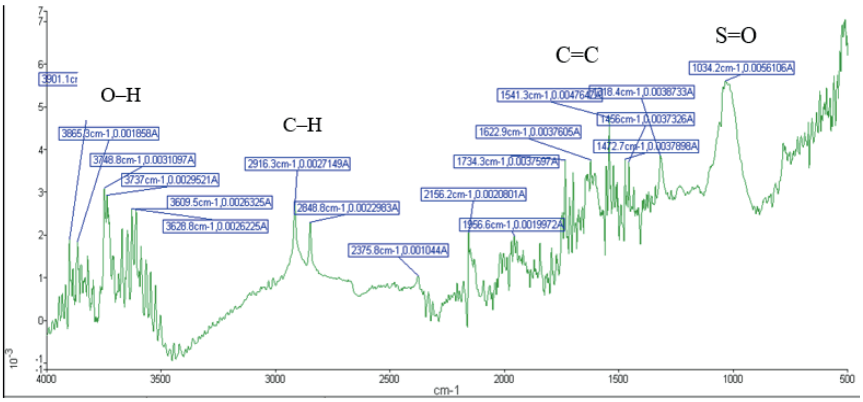

Figure 5. FT-IR absorbance spectrum of Eucalyptus globulus

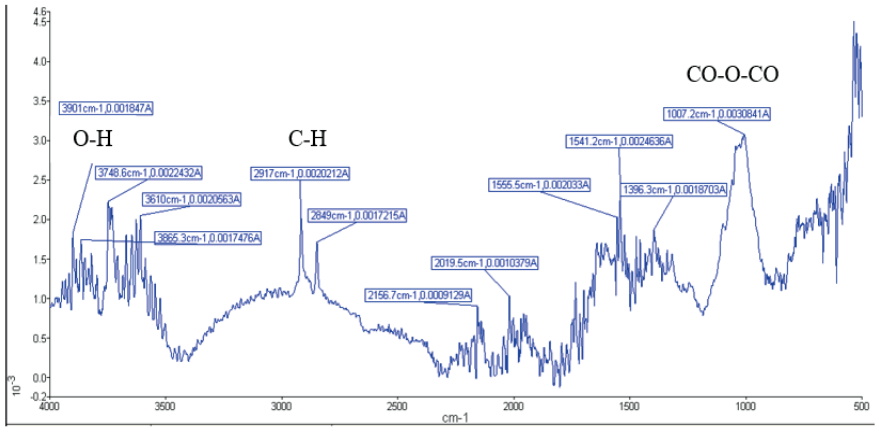

Figure 6. FT-IR absorbance spectrum of Justicia schimperiana

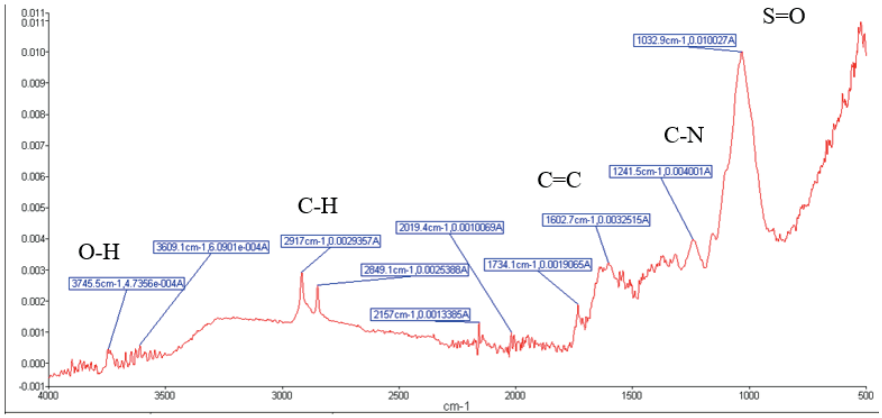

Figure 7. FT-IR absorbance spectrum of Croton macrostachyus

2849 cm-1 indicated the presence of C-H stretching of alkane. The peak at 1602 cm^{-1} attributed to C=C stretching of alkene and 1241 cm^{-1} attributed to C-N Stretching due to amaine functional groups. The broad peak at 1032 cm^{-1} is attributed to S=O stretching due to sulfoxide functional groups.

Adult Immersion Test

Adult immersion tests were conducted in order to identify anti-ectoparasite activity of medicinal herbal plants. As shown in Figure 8, after exposure to Amitraz 12.5% for 1 hour and P. *dodecandra* at concentrations of 50 mg/ml and 100 mg/ml for 6 hours, there was a substantial increase in tick mortality (40%). Less tick mortality (20%) was observed 6 hr post exposure with 12.5mg/ml and 25 mg/ml concentrations of P. *dodecandra* extract.

Figure 8 shows that after exposure to Amitraz 12.5% for 1 hour and P. dodecandra at concentrations of 50 mg/ml and 100 mg/ml for 6 hours, there was a substantial increase in tick mortality (40%). Less tick mortality (20%) was observed 6 hr post exposure with 12.5mg/ml and 25 mg/ml concentrations of P. *dodecandra* extract. At 24 hr

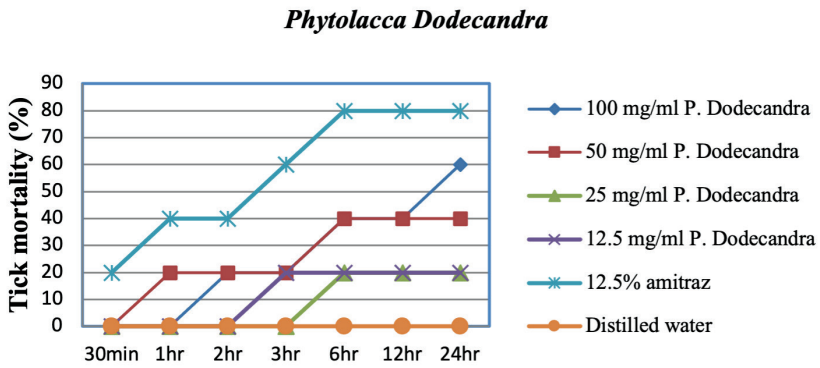

Figure 8. Mortalities of ticks at different time intervals in Phytolacca.Dodecandra extract.

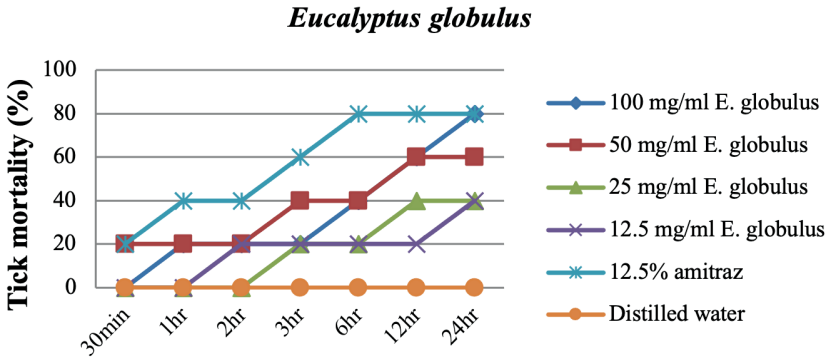

Figure 9. Mortalities of ticks at different time intervals in Eucalyptus globulus extract.

post exposure period Amitraz 12.5% (80%) and 100mg/ml (20%) P. *dodecandra* had the highest tick killing effect compared with the rest of the extract concentrations.

A substantial increase in tick mortality (60%) was observed after 3 hr post exposure with Amitraz 12.5% and 100 mg/ml concentration of E. *globulus* extract, and 6 hr exposure with 50mg/ml and 25 mg/ml concentrations of E. globulus extract (Figure 9). Moderate tick mortality (40%) was observed 6 hr post exposure with 12.5mg/ml, 25 mg/ml and 50mg/ml concentrations of E. globulus extract. Subsequently, after 24 hr post exposure, Amitraz 12.5%, 50mg/ml and 100mg/ml of E. *globulus* extract had the highest tick mortality (80%) effect than the rest of the extract concentrations.

As shown in Figure 10, a substantial rise in tick mortality (40%) began 3 hours after exposure to Amitraz 12.5% and at 6 hours after exposure with 50 mg/ml and 100 mg/ml concentration of J. *schimperiana* extract. At 24 hr post exposure, 50 and 100 mg/ml concentrations of the extract have caused significantly higher tick mortality (60%) than the rest of extract concentrations. At 24hr exposure, the 12.5mg/ml and 25 mg/ml has caused significant tick mortality (40%) than negative control (distilled water).

According to Figure 11, a substantial increase in tick mortality (40%) began 2 hours after exposure with Amitraz 12.5% and 6 hours after exposure with 50 mg/ml and 100 mg/ml concentrations of C. *macrostachyus* extract. After 24 hours exposure, 50 and 100 mg/ml concentrations of Croton *macrostachyus* extract caused

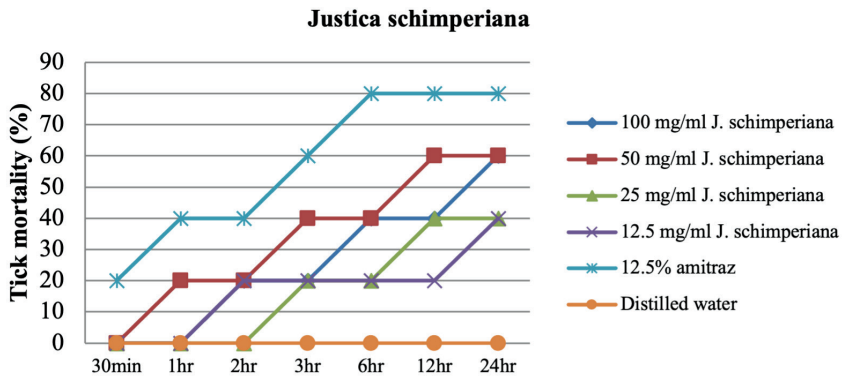

Figure 10. Mortalities of ticks at different time intervals in Justica schimperiana extract.

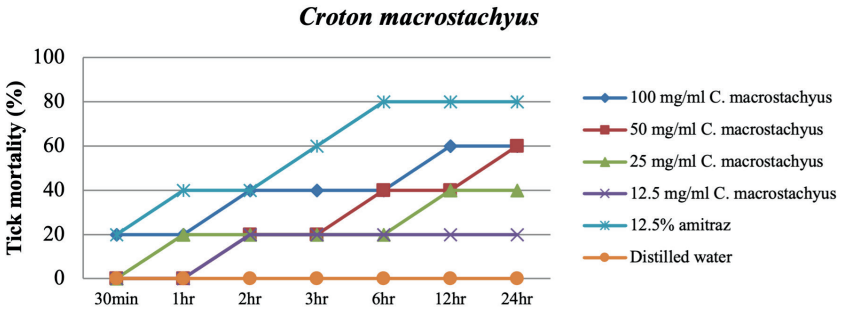

Figure 11. Mortalities of ticks at different time intervals in Croton macrostachyus extract.

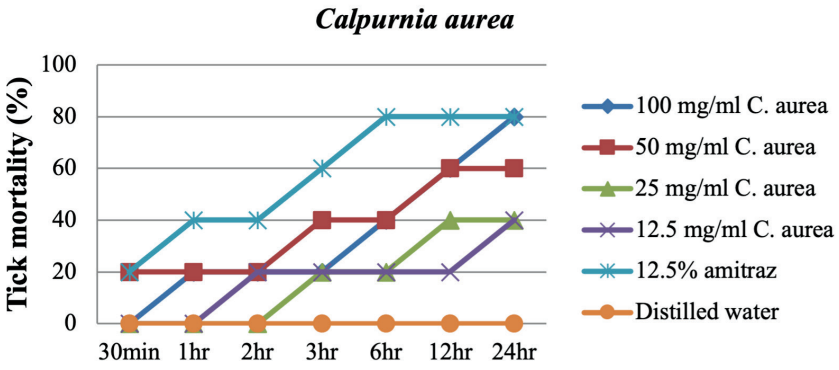

Figure 12. Mortalities of ticks at different time intervals in Croton aurea extract.

a substantial increase in tick mortality (to 60%) compared to the other concentrations. After 24hr exposure, 12.5mg/ml and 25 mg/ml concentrations of Croton *macrostachyus* extract has caused significant tick mortality (20%) than negative control (distilled water).

A significant increase (40%) in tick mortality started 1 hr post exposure with Amitraz 12.5% and 6 hr exposure with 50mg/ml and 100 mg/ml concentrations of C. *aurea* extract (Figure 12). When compared to 25 mg/ml and 12.5 mg/ml concentrations C. *aurea* extract, tick mortality was significantly higher at 24 hours post-exposure with 50 mg/ml and 100 mg/ml concentrations of C. *aurea* extract and Amitraz 12.5%. After 24 hours of exposure, the lowest concentration (12.5 mg/ml) of C. aura showed a substantial increase in tick mortality compared to the negative control (distilled water). The study finding revealed that Calpurinia *aurea* extract has a significant antiparasitic activity against small ruminant ticks. The current findings are similar with Gebrezgabiher et al,[42] Zorloni et al.[43] and Teklay et al.[44] who reported water extracts of Calpurinia *aurea* has insecticidal activity against ticks. Similarly, studies reported that, application of water-based extract of Melia *azedarach* L.,[45] Solanum *incanum* L,[46] Aloe excelsa A. Berger,[12] Nicotiana *tabacum* L., [47] Azadirachta indica A. Juss,[48] Otostegia integrifolia Benth,[49] Aloe megalacantha Bark,[44] Guizotia *scabra*,[50] Citrus *aurantifolia*,[51] Cassia *nigicans*[52] and Commiphora *erythraea*[53] have insecticidal activity against ticks.

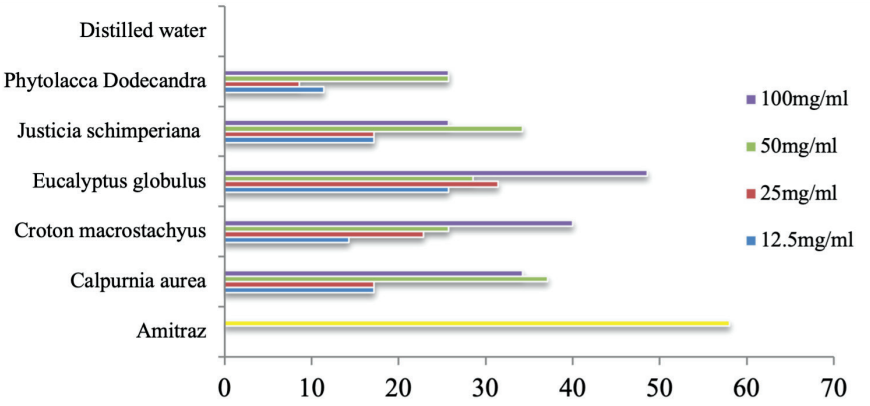

Figure 13. Mean mortalities of ticks at different concentration intervals in different medicinal plant extracts.

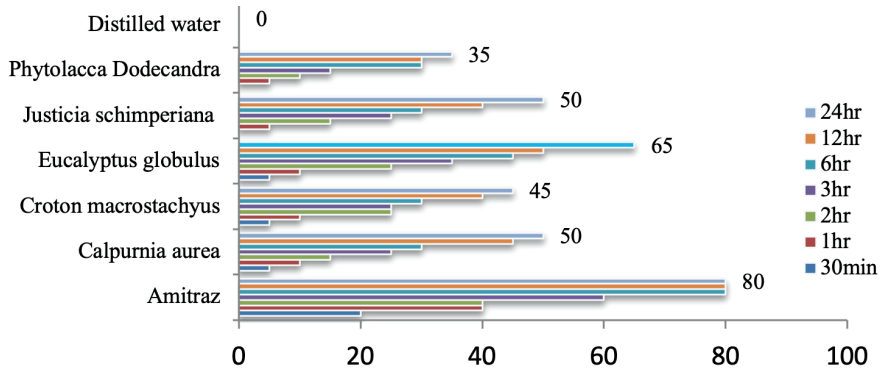

Figure 14. Mean mortalities of ticks at different time intervals in different medicinal plant extracts.

Table III

Two-way analysis of variance (ANOVA)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
plantonly$Plant_extract	4	3589	897.1	2.248	0.0672 .
Residuals	135	53886	399.2		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

As shown in Figure 13, Amitraz 12.5% had the highest tick mortality and followed by Eucalyptus *globulus*. Phytolacca *Dodecandara* showed the lowest tick mortality than the other extracts. The present study revealed that the mean mortality of ticks was increased significantly with increased dosage (concentration) in vitro treatment for the tested botanicals.

The results of the current investigation showed that using higher dosages (concentrations) of the investigated botanicals in vitro considerably increased the mean tick mortality, which were shown in Figure 14. This outcome is consistent with that of Adenubi,[54] who found that the dosages (concentration) and exposure duration had an impact on the mortality effect of plants.

As indicated in Table III and IV, the plant extracts have similar effects on morality rate of ticks and the mean mortality of ticks was increased significantly with increased concentration and exposure time after in vitro treatment for the tested plants.

Mode of Action

Few studies have been carried out to fully understand how these naturally occurring compounds act as anti-ectoparasites, and the mode of action of many compounds derived from plants used for ectoparasite control is not well studied.[55] Several studies report that, presence of phytochemicals (secondary plant metabolites) possess various biological activities including anti-parasitic, antioxidant, anti-bacterial, and antifungal.[56-59] In line with this, the presence of phytochemicals (active components) including phenols, flavonoids, alkaloids, tannin, saponin etc. in different plants, may be responsible candidates for the anti-ectoparasite properties (growth inhibition).[27, 60,61] According to de Souza Chagas,[62] phytochemicals may act to counter growth of regulatory hormones, limiting egg growth, causing dehydration, inhibition of breathing (clog airways) and prevent chitin formation. In general, the biology of how the plant essential oils affect ectoparasite remains unexplored and warrants an ideal opportunity to work further on the plant metabolites involved in the process.

Conclusion

The leather sector has a huge potential for employment, and export revenue generation in the world economy. Ethiopia has one of the largest livestock populations in the world. However, this huge resource potential is constrained and threatened by compound effects of ectoparasites, poor management and malnutrition. Using synthetic pesticides to treat ectoparasites has become a critical global issue due to the increasing resistance to pesticides of the ectoparasites and environmental pollution. This study revealed that P. *dodecandra*, E. *globulus*, C. *macrostachyus*, J. *schimperiana* and C.

Table IV

Mean and SD number of ticks mortality at of different time interval and concentration

Extract Concentration (mg/ml)	Mean and SD tick mortality at different concentration and exposure time						
	30min	1hr	2hr	3hr	6hr	12hr	24hr
100mg/ml	8 (10.9)	12 (10.9)	28 (10.9)	32 (17.8)	44 (8.9)	52 (10.9)	68 (10.9)
50mg/ml	4 (8.9)	12 (10.9)	20(0)	28 (10.9)	40 (0)	48 (10.9)	60 (14.1)
25mg/ml	0 (0)	4 (8.9)	8 (10.9)	20 (14)	24 (8.9)	40 (14.1)	40 (14.1)
12.5mg/ml	2.86 (7.5)	8.58(15.7)	17.15(13.8)	22.85(18)	28.57 (25.4)	28.5 (25.4)	34.3 (25)

aurea plants extract have shown good result in tick mortality after 24 h of exposure. Research on plant extracts for use in ectoparasite control has grown in recent years and the plants used in the study are a viable alternative to commercial acaricides.

Competing Interests

The authors declare that they have no competing interests.

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