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Research Article

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Lice Infestation and *In-vitro* Effect of *Datura stramonium*, *Opuntia ficusindica*, *Jatropha curcas* and *Ricinus comunis* on *Bovicola ovis*

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Abstract

Background: Louse infestations are the major threats to sheep production and productivity in Ethiopia. In Ethiopia, botanical surveys have shown that *Datura stramonium, Opuntia ficus indica, Jatropha curcas,*and *Ricinus cominus* are traditionally used to combat ectoparasites in livestock.

Methods: A cross-sectional and experimental study was conducted from November 2018 to April 2019 to estimate the prevalence of louse infestation, assess the risk factors, and evaluate *in-vitro* experiments on the louscidalefficacy of methanolic leaf extracts of *D. stramonium, O. ficusindica, J. curcas*, and petroleum ether seed oil extracts of *R. cominus* against *Bovicola ovis.*

Results:Of the total sheep examined, 109/384 (28.3%) were infested by *B. ovis*. The overall prevalence of lice infestation was statistically significant (p< 0.05)) with age and body condition score. The louscidal activity of the selected medicinal plants and a commercially available acaricide (0.1% diazinon) against *B. ovis* was tested *in vitro* at different time intervals using the in vitro adult immersion test at concentrations of 200, 100, 50, 25, 12.5, and 6.25 mg/ml. Crude leaf extracts of *D. stramonium, J. curcas,* and *R. cominus* seed oil caused high mortality of *B. ovis* at all concentrations, with a comparable effect to the activity of 0.1% diazinon within 24 hours of exposure. Moreover, a crude leaf extract of *O. ficusindica* produced 63% mortality of *B. ovis* within 24 hours of exposure at 200 mg/ml concentration. Crude leaf extracts of *D. stramonium* and *J. curcas* and *R. cominus* seed oil had strong louscidal activity at lower concentrations against *B. ovis.*

Conclusion: The present study showed that lice were the prevalent ectoparasites of sheep's in the area. The findings unequivocally demonstrated that three of the four plants extract, such as *D. stramonium, R. cominus* and *J. curcas* had potent louscidal activity comparable to the commercial acaricide (0.1% diazinon).

Keywords: Bovicola ovis; concentrations; In-vitro;Medicinal plants; Mortality

Background

The term "ectoparasites" refer to parasites that live on or burrow into the surface of host's epidermis[1]. Ectoparasites acquire blood meal from their by inserting the mouthparts into the integument of the host. However, there have been reports on ticks occurring in the subcuticular regions or deeper regions of the host skin [2]. The association between arthropod ectoparasites and hosts may take on a variety of forms. In some cases, the parasite may be totally dependent on the host feeding on the host, or they live only occasionally on the host, without being dependent on it[1].

Ectoparasites are also described as the predominant cause of pre-slaughter skin defects [2]. Skin problems caused by ectoparasites such as lice, keds, mange mites, ticks, and other skin diseases result in serious economic losses to smallholder farmers, the tanning industry, and the country as a whole. According to Kassa [4], skin problems due to ectoparasites cause 35% of sheep skin rejection and 56% of goat skin rejection per year. Skin defects and diseases are known to affect the quality of the skin. For instance, in 1996/97, six tanneries that are found in and around Addis Ababa rejected 2,037,745 pieces of skin, which caused a loss of 6.3 million USD [4], and in 1998/99, three tanneries that are found in Amhara regional state have reported 443,602 pieces of skin rejection per year, which is worth 1.4 million USD [5]. The infestation of lice on sheep and goats has been reported from different parts of Ethiopia. Lice were thought to be the cause of cockle-following keds because they are visible on the skin surface of affected animals, causing skin rejection [6].

In many African countries, including Ethiopia, the use of acaricides is the only method of controlling ectoparasites. It is cheaper and easier to attempt to kill the parasite. However, several reports have shown that the use of some of these acaricides is constrained by the rising development of resistance, high toxicity, and environmental concerns [7]. In addition, control methods based on the application of synthetic acaricides sometimes fail to keep the number of ectoparasites below economic threshold levels. It is therefore necessary to find potential alternatives that can minimize the negative effects of synthetic acaricides. The identification of novel active plant-derived natural compounds could increase the number of available chemotherapeutic agents by reducing the frequency of the development of resistance and providing alternative drugs with greater acceptance, especially in terms of environmental safety [8].

It is obvious that in far-off rural parts of Ethiopia, synthetic acaricides and other drugs are not affordable to the majority of poor livestock owners. Even when accessible, the farmers tend to treat their livestock with synthetic products in an unscientific way and illegally import them at a high cost from neighboring countries. Furthermore, livestock owners frequently complain about the poor killing effect of most existing acaricidal drugs. As a result, livestock keepers continue to rely on endogenous ethnoveterinary knowledge and practices for socially acceptable, inexpensive, and locally available remedies for managing lice and other ectoparasites.

In Ethiopia, botanical surveys have shown that *D. stramonium, O. ficusindica, J.curcas,* and *R. cominus* are traditionally used against ectoparasites of livestock in ethno-veterinary practices [9-11]. However, limited researches have been done to explore the *in-vitro* and *in vivo* effects of such selected medicinal plants against sheep lice. Herewith, we hypothesized that since these plants have been used traditionally by the livestock keeping community, their extracts have good acaricidal activity against commonly prevalent lice. We also found it very essential to undertake a thorough killing efficacy evaluation of botanicals traditionally used by the owners of livestock as an alternative lice control strategy. Therefore, the objectives of this study wereto estimate the prevalence and associated risk factors of lice infestation in sheep, determine the secondary metabolites present in each study plant

extract, and evaluate the *in-vitro* louscidal activity of methanolic and petroleum ether extracts of the selected medicinal plants against *B. ovis.*

Methods

Study area. The prevalence study was conducted in the Haramaya district. The in-vitro louscidal evaluation and phytochemical screening tests were carried out at the parasitology and biochemistry laboratories of Haramaya University College of Veterinary Medicine in Haramaya, which is located in Oromia Regional State's eastern Hararghe Zone, 508 kilometers from Addis Abeba. It is located at an altitude range of 1800 to 2345 (an average 2047) m.a.s.l, 9 26'N latitudes and 42 3'E longitudes. The mean annual rainfall is 780 mm. The mean annual minimum and maximum temperatures are 8.5oC and 24.4oC, 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 population and sampling method. The study population was local sheep that were reared in and around the Haramaya district. A cross-sectional study was conducted on 384 randomly selected sheep. The sample size was determined based on the expected prevalence of 50% and the absolute desired precision of 5% at a 95% confidence level. The desired sample size was calculated using the standard formula described by Thrusfield [12].

Study design.A cross-sectional study was carried out from November 2018 to April 2019 to estimate the prevalence and associated risk factors of sheep's louse, whereas an experimental design was applied to evaluate *in-vitro* on the louscidal efficacy of methanolic leaf extracts of *D. stramonium, O. ficusindica, J. curcas,* and petroleum ether seed oil extracts of *R. cominus* against *B. ovis.* The phytochemicals present in each plant extract were also assessed using standard procedures.

Lice collection and identification methods. Adult sheep lice, *B. ovis*, were collected from sheep for the in-vitro louscidal efficacy test using four selected medicinal plants. The coat-brushing technique was used for the collection of lice from sheep. The parasites were maintained in plastic cups, into which water-soaked cotton was placed to increase the humidity of the air in the cups. The cups were covered with gauze to allow the free circulation of air into the cups, and then the parasites were transported to Haramaya University's parasitology laboratory. Identification and recording of the parasites were conducted within a few hours of collection under a stereoscopic microscope, according to Wall and Shearer [1], and only adult lice were used in this experiment.

Plant material collection and extraction. The mature and well-developed leaves of the plant species *J. curcas, D. stramonium, and O. ficusindica,* as well as the seeds of *R. cominus,* were selected based on the information obtained from previous botanical surveys [9–11]. People have traditionally used these plants for the control of ectoparasites. The selected plants of *J. curcas* (leaf), *D. stramonium* (leaf), and *O. ficusindica* (leaf) were collected from eastern Hararage Zone Jarso, Haramaya, and Gursum districts, respectively, while mature *R. cominus* (seed) was collected from Babile district in eastern Hararage. The plants were identified and verified at Haramaya University College of Agriculture with voucher No FENOV/11/09, FENOV/74/06, FENOV/22/11, and FENOV/95/05, respectively.To reduce possible contamination, principally by fungi, latex gloves were worn when leaves were collected. The collected plant material was spread out on paper sheets in the shade at room temperature to dry for around two weeks. When desiccated, all selected plant leaves were stored in sealed containers separately for extraction. The dried plant materials were crushed in an electric grinder into a coarse powder. For each plant, one hundred grams (100 g) of powdered material were soaked in four hundred ml (400 ml) of methanol separately for 48 hours on an orbital shaker. Each extract was 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 petroleum ether seed extract of *R. cominus* oil in the soxhlet apparatus [13]. The mature *R. cominus* seeds were powdered using a mortar and pestle. The powdered seeds (200 g) were extracted by (Soxhlet) with petroleum ether (boiling point 60–80^{o}C) for 6 hrs. The solvent after extraction was removed by distillation in a water bath [14].

The extraction rates (%) were calculated in accordance with Eloff [13] as shown below:-

$$\text{Extraction rate (\%)} = \frac{\text{Extracts weight (g)}}{\text{Plant material weight (g) before extraction}} \times 100$$

Preliminary phytochemical screening of solvent extracts. The crude methanol leaf extracts and petrolium ether extract seed oil were screened for the presence or absence of secondary metabolites such as alkaloids, flavonoids, steroidal compounds, phlobatannins, saponins, tannins, phenolic compounds, triterpens and glycosides using standard procedures [15, 16].

***Test for saponins*:** The extract was diluted with an appropriate solvent and made up to 20 ml. The suspension was then shaken in a graduated cylinder for 15 minutes, forming a 2 cm layer of foam.

***Test for tannins*:** 2 ml of diluted sample was treated with 3 drops of 10% ferric chloride and formation of bluish-black color indicates presence of tannins.

***Test for steroids*:** The test for steroids was done by the Liberman acid test. A portion of the extract was treated with drops of acetic anhydride. Concentrated H_2SO_4 was carefully added to the side of the test tube.

Test for flavonoids: 5 ml of dilute NH3 solution was added to 3 ml of the test sample, followed by the addition of 2 ml of concentrated H2SO4.

***Test for phenolic compounds*:**1 ml of test solution was treated with 10% ethanolic ferric chloride, and a color change to dark blue is affirmative of the positive test.

***Test for alkaloids*:** 1 ml of test solution was treated with a few drops of Dragendrof's and Mayer's reagent and formation of precipitate indicates a positive result.

***Test for glycosides*:** 1ml of the test solution was mixed with 1 ml of glacial acetic acid and then treated with one drop of 5% ethanolic ferric chloride solution. 1 ml of concentrated H_2SO_4 was carefully added down the side of the test tube. Appearance of a brownish ring between the two formed layers shows positive result.

Crude oil extracts were considered positive for the froth formation indicated the presence of saponins. While green precipitate indicates the presence of tannins. Whereas, the upper layer turns red and should yellow with green fluorescence indicates the presence of steroids. The presence of flavonoids was indicated by red or orange color formation. Green to black precipitate indicates the presence of phenolic compound. Occurrence of orange-red precipitate indicates the presence of alkaloids while the appearance of red color shows the presence of triterpenes. Moreover, the deposition of a red precipitate was taken as an evidence for the presence of phlobatannins [15, 16].

In vitro louscidal efficacy test. For the louscidal efficacy test, adult immersion test was used as described by Ghosh*et al.* [17]. The dried extracts of *D. stramonium*, *J. curcas* and *O. ficusindica* were diluted in distilled water, and *R. cominu s*oil was diluted in 2% Tween 80 at the different concentrations required for the bioassays (20%, 10%, 5%, 2.5%, 1.25% and 0.65%). The *in-vitro* tests were started within one hour after lice were collected [18]. Ten active lice in three replications for each concentration of study plant extract were put into plates. That means 30 lice for each concentration of study plant extract making a total of 240 lice for each study plant extract then a total of 720 lice were used for this study. 1 ml of each

concentration were directly added to the three replicate plates and incubated at 27-28°C and 75-80% relative humidity for 24 hrs. These three replicates were treated with distilled water or 2% Tween 80 as negative and 0.1% diazinon 60 EC as positive controls [19] which were performed for each extract. *In vitro* louscidal efficacy test for each study plant extract were done on different day. The test solutions, treated and controls, were removed just after one-minute contact time using whatman No. 1 filter paper. Each louse in each plate was closely observed for any death under stereomicroscope at different time intervals: 30 min, 1 hr, 2 hrs, 3 hrs, 6 hrs and 24 hrs [20].

The deaths of lice were strictly followed. If any signs of life such as movement of antennae, gut cells or minimal legs movements were observed with stimulation by needle, then the lice were categorized as alive. The lice were judged as dead, only if there were no signs of movement at all [19]. The number of lice deaths was recorded in pre-prepared format. The percent mortality rates of lice were calculated as per Pamo *et al.* [21].

$$\text{Corrected mortality} = \frac{\%\ \text{Treated mortality} - \%\ \text{Negative control mortality}}{100 - \%\ \text{Negative control mortality}} \times 100$$

Mortality in the plates treated with crude extracts of plant parts was corrected to take account of control mortality using Abbott's correction. Louscidal effects were classified as: strong if mortality was >80%; moderate if mortality was 80–60%; weak if mortality was 60–40%; and little or no activity if mortality <40%.

Data analysis: The collected raw data were stored in a Microsoft Excel spreadsheet following the edition. A statistical software package, SPSS for Windows, version 17.0, was used for data analysis. A chi-square test was used to determine the presence of an association between the prevalence of louse infestation and the study area, ruminant species, sex, age, breed, body condition score, and production system. Analyses of variance (one-way ANOVA, Tukey multiple comparisons test) were used to compare the means of mortality ± standard error of different treatments (concentrations) of the extracts and controls in different time periods used for *in vitro* efficacy studies of medicinal plants. The level of significance was set at $P < 0.05$ with a 95% confidence interval.

Results

Of the total sheep examined,109/384(28.3%) were infested by *B. ovis*. Among the risk factors investigated, sheep age and body condition had a significant effect on the occurrence of louse infestation($p<0.05$) (Table 1).

Table 1 The overall prevalence of *B. ovis* based on the various risk factors assessed.

Risk factors	Categories	No. examined animal	No. positive animal	Prevalence	χ^2	P- value
Sex	Female	210	65	31	1.652	0.199
	Male	174	44	25.3		
Age	Young	174	41	23.5	19.43	0.001
	Adult	210	68	32.4		
Body condition	Poor	129	64	49.6	39.90	0.001
	Medium	163	33	20.3		
	Good	92	12	13.4		
	Total	384	109	28.3		

Physicochemical characteristics yield and chemical analysis of different plant extracts: Physical characteristic features of extracts and percentage yield have been depicted in Table 2. Chemical analysis of different plant extracts contains alkaloids, saponins, phlobotanin, steroids, flavonoids, glycosides, tannins, and triterpens (Table 3).

Table 2Physical characteristics and percentage yield of crude/oil extracts of the study medicinal plants.

Plants	Plant part extracted	Solvents for extraction	Weight ofdry powder (g)	Weight of dry extract (g)	Yield %	Means of dilution
D. stramonium	Leaf	Methanol	1000	156	15.6	Distilled water
J. curcas	Leaf	Methanol	1000	223	22.3	Distilled water
O. ficusindica	Leaf (Latex)	Methanol	500	45	4.5	Distilled water
R. cominus	Seed	Petroleum ether	1000	120	12	2% Tween80

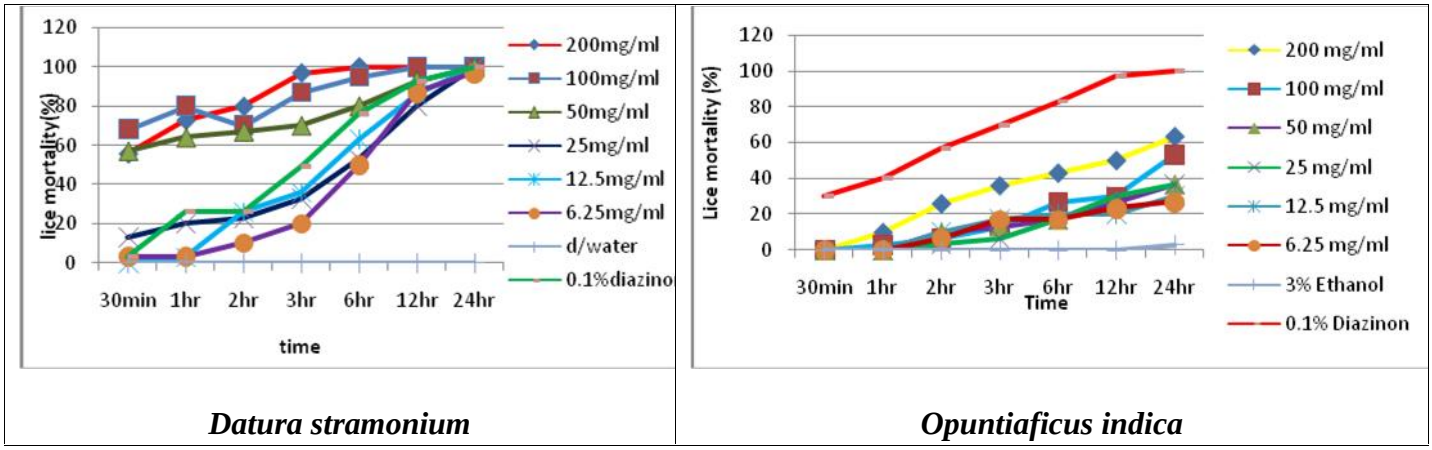
Table 3Qualitative determinations of active ingredients in crude/oil extract of the study plants

Secondary metabolites	*D. stramonium*	*O. ficusindica*	*R. cominus*	*J.curcas*
Saponin	+	+	+	-
Tannin	+	+	+	+
Phenolic cpds	+	+	+	+
Steroids	+	-	+	+
Flavonoids	+	+	+	+
Phlobotanin	+	-	-	ND
Glycosides	+	-	+	-
Triterpens	-	-	+	+
Alkaloids	+	-	+	-

Source: '+' Positive; '-' Negative; 'ND' Not done

Evaluation of in-vitro louscidal activity: *Datura stramonium* crude leaf extract at 100 mg/ml concentration demonstrated higher louscidal activity (68% efficacy) after 30 minutes of exposure. After 6 hrs of exposure, a 200 mg/ml concentration of *D. stramonium* leaf extract showed 100% mortality, which was significantly higher ($P < 0.05$) as compared to other concentrations except for 100 mg/ml, which showed 95%. After 24 hours of exposure, with the exception of the lowest concentration (6.25 mg/ml), all test concentrations caused 100% mortality of lice. Moreover, there was no statistically significant difference ($P > 0.05$) in the louscidal activity among different concentrations after 24 hrs of exposure when compared to the reference drug (0.1% diazinon) (Figure 1).

All concentrations of *O. ficusindica* leaf extracts showed no louscidal activity at 30 minutes, but, a higher mortality percentage of up to 50% was recorded on lice exposed to 200 mg/ml concentration at 12 hrs. There was no statistically significant difference in louscidal activity across all concentrations of *O. ficusindica* extract ($P > 0.05$). Furthermore, when compared to the positive control (0.1% diazinon), O. ficusindica had significantly lower louscidal activity ($P < 0.05$).).When compared to the reference drug (0.1% diazinon), concentrations of 200 mg/ml and 100 mg/ml resulted in mortality rates of 63% and 53%, respectively, after 24 hours of exposure, which was statistically significant($P < 0.05$) (Figure 1).

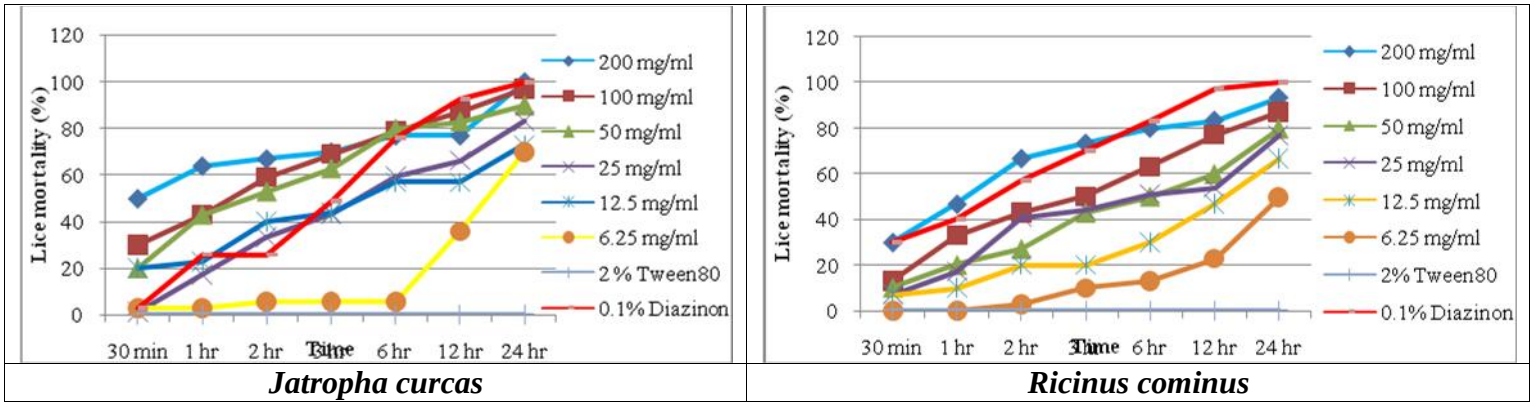


Datura stramonium ***Opuntiaficus indica***

Figure 1 Mortality of *B. ovis*treated with crude extracts of *Daturastramonium* (leaf) and *O. ficusindica* (leaf)

Jatropha curcas leaf extract showed higher louscidal activity (33%) at the concentration of 200 mg/ml after 30 minutes of exposure which was significantly higher mortality ($P< 0.05$) as compared to all <100 mg/ml concentrations. After 12 hrs of exposure, 200 mg/ml concentration of *J. curcas* leaf extract showed 83% mortality which was statistically not significant ($P> 0.05$) as compared with other concentrations except at 12.5 mg/ml and 6.25 mg/ml. While after 24 hrs exposure, 200 and 100 mg/ml concentrations of *J. curcas* crude leaf extract caused mortality of 93% and 87% respectively which was statistically insignificant ($P> 0.05$) when compared to the reference drug (0.1% diazinon) (Figure 2).

Ricinus cominus seedoil extract at 200 mg/ml concentration showed higher louscidal activity (50%) after 30 minutes of exposure which was significantly higher ($P< 0.05$) as compared to 25, 12.5 and 6.25 mg/ml concentrations. After 24 hrs of exposure 200 mg/ml concentration of *R. cominus* seed oil extract showed 100% mortality which was significantly higher ($P< 0.05$) as compared to the lower concentration of 6.25 mg/ml. Moreover, there was no statistically significant difference ($P> 0.05$) in the louscidal activity among different concentrations after 24 hours exposure except at lower concentration (6.25 mg/ml) when compared to the reference drug (0.1% diazinon) (Figure 2).



Jatropha curcas ***Ricinus cominus***

Figure 2Mortality of *B. ovis* treated with crude extracts of *J. curcas* (leaf) and *R. cominus* oil (seed)

Table 4Louscidal activity comparisons of different extracts on *B. ovisafter* 30 min and 1 hr exposure to the extract

Dose (mg/ml)	Mean number of lice dead (mean of mortality ± SE) post exposure					
	30 min			1hr		
	D.stramonium	***J. curcas***	***O. ficusindica***	***D.stramonium***	***J. curcas***	***O. ficusindica***
200	5. 67±2.33^{a}	3.00±.577^{a}	00±.000^{a}	7.33±1.763^{a}	4.67±333^{ab}	1.00±.577^{b}
100	7.00±.577^{a}	1.33±.333^{b}	00±.000^{b}	8.00±.577^{a}	3.33±.333^{ab}	33±.333^{b}
50	5.67±.666^{a}	1.00±.577^{b}	00±.000^{b}	6.33±.333^{a}	2.00±.577^{b}	.00±.000^{c}
25	1.33±.666^{a}	667±.333^{a}	00±.000^{a}	1.33±.1.33^{a}	1.67±.1.66^{a}	.00±.000^{b}
12.5	00±.000^{a}	667±.333^{a}	00±.000^{a}	33±.333^{a}	1.00±.577^{a}	00±.000^{a}
6.25	.33±.333^{a}	00±.000^{a}	00±.000^{a}	33±.000^{a}	1.00±.666^{a}	00±.000^{a}
-ve control	000±.000^{d}	000±.000^{d}	000±.000^{d}	000±.000^{d}	000±.000^{d}	000±.000^{d}
+ve control	333±333$^{a.}$	2.33±1.53^{a}	2.33±.881^{a}	1.66±333^{c}	4.00±1.00^{c}	4.00±1.00^{c}

Mean values with different letters in the same column are significantly different ($P< 0.05$).

Table 5: Louscidal activity comparisons of different concentrations of crude extracts on *B. ovisafter* 2 and 3 hrs exposure

Dose (mg/ml)	Mean number of lice dead (mean of mortality ± SE) post exposure					
	2 hr			3 hr		
	D.stramonium	***J. curcas***	***O.ficusindica***	***D.stramonium***	***J. curcas***	***O.ficusindica***
200	7.33±1.763^{a}	1.00±.573^{ab}	1.00±.577^{b}	9.67±.333^{a}	6.67±.333^{b}	3.67±.333^{c}
100	8.00±.577^{a}	3.33±.333^{b}	.33±.333^{c}	8.67±.333^{a}	5.00±.577^{b}	1.33±.667^{c}
50	6.33±.333^{a}	2.00±.577^{b}	00±.000^{b}	7.33±.666^{a}	.33±.667^{b}	4.33±.333^{c}
25	1.33±.333^{a}	1.667±333^{b}	00±.000^{b}	2.67±.666^{a}	4.33±.667^{a}	67±.333^{b}
12.5	.33±.333^{a}	1.00±577^{a}	00±.000^{a}	4.67±.333^{a}	1.67±1.202^{a}	2.00±1.000^{a}
6.25	. 00±.000^{b}	667±.666^{b}	00±.000^{b}	1.67±.881^{b}	1.67±1.667^{b}	1.00±.577^{b}
-ve control	..000±.000^{d}	. .000±.000^{d}	.000±.000^{d}	.000±.000^{a}	.000±.000^{a}	.000±.000^{a}
+ve control	.1.666±333^{c}	6.667±1.452^{c}	6.667±1.453^{c}	4.000±.577^{a}	9.333±.667^{b}	9.333±.667^{b}

Table 6 Loucocidal activity comparisons of different concentrations of crude extracts on *B. ovis after* 6 and 12 hrs exposure

Dose (mg/ml)	Mean number of lice dead (mean of mortality ± SE) post exposure					
	6 hr			12 hr		
	D.stramonium	***J. curcas***	***O.ficusindica***	***D.stramonium***	***J. curcas***	***O. ficusindica***
200	10.00±.000^{a}	7.33±.667^{b}	4.33±.333^{c}	10.00±000^{a}	577±.3333^{b}	5.00±000^{c}
100	9.00±.577^{a}	6.33±.881^{a}	2.67±.333^{b}	10.00±.000^{a}	7.67±.882^{b}	3.00±.000^{c}
50	8.33±.667^{a}	5.00±.577^{b}	1.67±.882^{c}	9.33±666^{a}	6.00±0000^{b}	2.67±.333^{c}
25	4.67±1.333^{a}	5.00±.000^{a}	1.67±.667^{a}	7.33±.666^{a}	5.33±.333^{ab}	3.00.000^{b}
12.5	6.33±881^{a}	3.00±1.154^{a}	2.00±1.000^{a}	8.67±.666^{a}	4.67±..333^{b}	2.00±1.000^{b}
6.25	5.00±577^{a}	1.33±.881^{a}	1.67±1.666^{a}	8.67±.666^{a}	2.33±1.452^{ab}	2.33±1.201^{b}
-ve control	000±.000^{c}	. 000±.000^{c}	. 000±.000^{c}	333±333^{d}	000±.000^{d}	. .0000±.000^{d}
+ve control	7.667±1.859^{b}	9.333±.667^{b}	9.3333±.6667^{b}	9.333±.333^{a}	9.666±.333^{a}	9.666±.333^{a}

Mean values with different letters in the same column are significantly different ($P< 0.05$).

Table 7 Comparisons on loucocidal activity of different concentrations of crude extracts on *B. ovi* safter24 hrs exposure

Dose (mg/ml)	Mean number of lice dead (mean of mortality ± SE) post exposure		
	D. stramonium	***J. curcas***	***O. ficusindica***
200	10.00±.000^{a}	8.00±.577^{b}	6.33±.333^{c}
100	10.00±.000^{a}	8.33±.333^{b}	5.33±.333^{c}
50	10.00±.000^{a}	8.00±5.515^{b}	3.67±333^{c}
25	10.00±.000^{a}	7.67±.577^{b}	3.66±.333^{c}
12.5	10.00±.000^{a}	6.67±333^{ab}	3.00±1.527^{b}
6.25	9.67±.333^{a}	5.00±577^{b}	2.67±1.201^{b}
-ve control	1.000±.000^{b}	.666±333^{b}	333±333^{b}
+ve control	10.000±.000^{a}	9.666±.333^{a}	9.666±.333^{a}

Table 8 The corrected mortalities of *B. ovis* exposed to different concentrations of petroleum ether extracts of *R. cominus* seed.

Dose (mg/mL)	Mean number of lice dead (mean of mortality ± SE) post exposure						
	30 min	**1 hr**	**2hr**	**3 hr**	**6 hr**	**12 hr**	**24 hr**
200	5.00±.577^{a}	1.33±. 333.a	.33±.333^{a}	.33±.333^{ab}	67±.333^{c}	.00±.000^{a}	2.33±.882^{d}
100	3.00±.577^{ab}	1.33±.333^{ab}	1.67±.333^{ab}	1.00±.000^{ab}	1.00±.577^{c}	.67±.333^{ab}	1.00±.000^{d}
50	2.00±.577^{abc}	2.33±.667^{ab}	1.00±.577^{abc}	1.00±.577^{ab}	1.67±.333^{c}	.33±.333^{abc}	67±.667^{d}
25	67±.667^{bcd}	1.00±.577^{abd}	1.67±.333^{abcd}	1.00±.577^{ab}	1.67±.333^{c}	67±.333^{abcd}	1.67±1.202^{d}
12.5	.33±.333^{cde}	00±.000^{abde}	33±.333^{abcde}	00±.000^{ab}	00±.000^{c}	3.00±1.000^{e}	3.67±333^{d}
6.25	2.00±.1.00^{cdef}	33±.333^{abdef}	1.67±.333^{abcde}	.33±.333^{ab}	1.33±.333^{c}	.00±.000^{abcdf}	1.33±.882^{d}
2% Tween80	.00±.000	00±.000	.00±.000	.00±..000	00±.000	.33±.333	33±.333
0.1% diazinon	3.00±.577^{cdef}	1.00±.577^{abdef}	1.67±.333^{ace}	1.33±.882^{ab}	1.00±1.000^{c}	1.33±.667^{abcdf}	67±.333^{d}

Mean values with different letters in the same column are significantly different ($P< 0.05$).

Comparative *in-vitro* louscidal activity of crude leaf extracts of *D. stramonium,J. curcas* and *O. ficusindica* at 6 hrs, 12 hrs and 24 hrs has been given in table 4-7. The results revealed that 100 and 50 mg/ml crude leaf extracts of *D. stramonium* showed statistically significant high louscidal activity ($P< 0.05$) after 6 hrs of exposure than *J. curcas*and *O. ficusindica*. In addition, 100 and 50 mg/ml crudes leaf extract of *D. stramonium* exhibited statistically significant highlouscidal activity ($P< 0.05$) after 12 hrs f exposure than *J. curcas* and *O. ficusindica* while after 24 hrs exposure except 200 mg/ml and 6.25 mg/ml concentrations of *J. curcas* and all concentrations of *D. stramonium* crude leaf extracts had statistically significant higher louscidal activity ($P< 0.05$) than *J. curcas* and *O. ficusindica* crude leaf extracts. In addition, *J. curcas* after 24 hrs of exposure showed statistically significant higher louscidal activity ($P< 0.05$) than *O. ficusindica* at 200 100, 50 and 25 mg/ml concentrations. However, because of the different extraction methods used, *R.cominus* effect is not included in comparison.

The *in-vitro* mean mortality values ± standard errors of mean of *B. ovis* at different time post exposure with varied concentrations of petroleum ether extracts of *R. cominus* seed was shown (Table 8). The result indicated that petroleum ether extracts of *R. cominus* seed showed 2.33±.882 and 1.00±.000 mean mortality effect after 24 hrs of exposure to 200 and 100 mg/ml concentrations, respectively (Table 8).

Discussion

The current study revealed that the overall prevalence of lice infestation was 28.3%. The age and body condition scores of animals were statistically significant ($p< 0.05$) and associated with the overall prevalence of lice infestation in the study area. The result is lower than that reported by [22], who reported a prevalence of 49.5% in sheep from Asella and its surroundings and 57% in sheep in Gondar [23]. This result, however, is higher than the prevalence recorded in Tigray, which is 1.3% [24], and Bahir Dar, which is 3.8% [25]. Such differences in prevalence may arise from differences in agro-climate, in the season during which the study was conducted, and in the management and health care of small ruminants in the study areas [26].

The result in the prevalence of *B. ovis* between sexes revealed a slightly higher rate in females than males, but no significant variation was observed; In the present study, the age-wise analysis revealed that there was a significant difference in prevalence between age groups, though the highest infection rate was recorded in adults rather than in young people. This result is comparable with the previous work by [27], who concludes that there is a difference in prevalence rates between age groups and that a higher infection rate was observed in adults than in young groups. This study also discovered a strong link between sheep body condition and louse infestation among body condition scores; *B. ovis* is more common in poor body condition animals. This result is similar to the previous study reported by [28], and the possible suggestion in this finding could be that adult and poorly conditioned animals were least resistant to ectoparasites infestation and lacked sufficient body potential to build resistance [29].

In the present study, among all four medicinal plants, *O. ficusindica* yielded a high percentage of extract (22.3%) (Table 2). The percentage yields of the extracts of *D. stramonium* and *R. cominus* were 15.6% and 12%, respectively (Table 2). However, there were no reports on the extraction of either of these plants in Ethiopia. The leaf extract of *J. curcas* produced a 4.5% yield. This finding is supported by [30, 31], who reported 17.7 % and 7.455% yields, respectively. In the present study, differences in the percentage yield of extracts among the four selected study plants might be due to variations in the nature of the plant species, the solvents used, and the method of extraction employed. In support of this, [32] described that the solubility of various plant ingredients depends on the extraction methods and the solvent used. This finding is in agreement with [33] who reported the presence of alkaloids, tannins, flavonoids, and saponins from the methanol leaf extract of *D. stramonium*. The

present finding also revealed that crude leaf extract of *O. ficusindica* was positive for saponins, tannins, flavonoids and phenolic compounds while negative for phlobotanin, glycosides, alkaloids, steroids, and triterpenes. Others [31] have reported that methanolic leaf extract of *O. ficusindica* was positive for saponins, tannins, flavonoids, and phenolic compounds, which is consistent with this finding.

The chemical analysis in this study revealed that the seeds of R. cominus were positive for alkaloids, glycosides, saponins, tannins, phenolics, flavonoids, triterpens, and steroid compounds but negative for phlobotanin. This finding is consistent with [34], who reported a nearly identical result regarding the constituents of R. cominus. The chemical analysis of the methanolic leaf extract of J. curcas found positives for tannins, triterpens, phenolic compounds, flavonoids, and steroids but negatives for saponins, glycosides, and alkaloids. This finding is similar to that of the study, which stated that the ethanolic extract of J. curcas seeds had a high content of unsaponifiable matter, sterols, and triterpene [35]. In contrast to the present findings, [36] noted the presence of alkaloids and saponins in J. curcas leaves. In addition, [30] reported that the acetone extract of J. curcas (seed) contained saponins but not triterpenes and steroids.

Datura stramonium crude extract and *R. cominus*seed oil demonstrated significant louscidal activity, with 100% mortality. *J. Curcas*, on the other hand, exhibited only minor louscidal activity. *O. ficusindia* also had a lower level of louscidal activity. However, the available scientific research on these plants' cidal effects is limited to lice. [17] reported that 95% ethanolic extracts of *D. stramonium* caused 20% mortality, within 72 hrs of treatment in an adult immersion test against Rhipicephalus (Boophilus) microplus. This finding is consistent with the current study, which shows that, when compared to other plants, *D. stramonium*has high louscidal activity comparable to diazinon. The potency of the extracts was concentration- and exposure-time dependent. This difference in mortality percentage might be due to variability in the amount or composition of secondary metabolites present in the plants at the time of extraction, as, the effectiveness of the plants depends on a complex mixture of secondary metabolites, such as alkaloids, glycosides, terpenoids, and flavonoids [37].

When the current findings are compared to previous studies, they show a mortality percentage variation. [38] reported, for example, that the chloroform fraction of *R. cominus* caused more than 50% lice mortality against L. vitul as early as 12 and 24 hours of exposure. Similarly, the methanolic leaf extract of the same plant showed insecticidal activity against *C. chinensis*[39]. *Jatropha curcas* crude extract showed comparable efficacy with the positive control (reference drug) against *B. ovis.* The current finding contradicts that of [40], who found that an ethanolic leaf extract of J. curcas had no cidal effect on adult Rhipicephalus annulatus engorged female ticks at a concentration of 100 mg/ml. This difference might be due to the difference in ectoparasite species, physiological status of ticks, and extraction solvent used among the studies [40].

Comparative *in-vitro* louscidal activity of different crude extracts of the four plants revealed that *D. stramonium,R. cominus* and *J. curcas* at 200 and 100 mg/ml concentrations and 0.1% diazinon demonstrated the highest *B. ovis*mortality of about 87–100% at 24 hours post-exposure, whereas *O. ficusindica* at all concentrations showed the least activity. Moreover, *D. stramonium* has shown extraordinary qualities in that it has achieved 100% mortality of lice even at concentrations as low as 12.5 mg/ml. The difference in efficacy might be due to variability in the biochemical composition of the plant materials. In this regard, assessment of secondary metabolites in plant extracts has revealed that the best active plant, *D. stramonium* has steroids, flavonoids, alkaloids, phlebotomine, and glycosides whereas the least active plant (*O. ficusindica*) didn't constitute the first three secondary metabolites. Lower louscidal activity of O. ficusindica could thus be attributed to a lower quantity of secondary metabolites and the absence of alkaloids, as higher parasite mortality was caused by a complex mixture of

secondary metabolites, as described by [41]. The highest mortality of *B. ovis*was observed by *D. stramonium* which is considered the best louscidal agent among the investigated and compared plants. The relative efficacy of *R. cominus*is not compared to the other medicinal plants in the study because of the difference in the extraction solvent used.

Conclusion

The present study identified lice as the prevalent ectoparasite of sheep in the area. The findings unequivocally demonstrated that three of the four plant extracts, such as *D. stramonium*, *R. cominus*and *J. curcas* had potent louscidal activity comparable to the commercial acaricide (0.1% diazinon). *D. stramonium*had the most potent louscidal effect of the three plants tested, even at low concentrations. The effect of *O. ficusindia* on *B. ovis*was minimal at a lower time in contact with the *B. ovis*and suggesting that it lacks essential elements to kill the lice. Further research into the safety and in-vivo efficacy, as well as the cost-effectiveness, of the products that demonstrated strong louscidal activity is required in order to replace conventional synthetic acaricide drugs.

Ethical Consideration

Ethics approval was not required for this study. This study involved only sheep for lice collection to observe the infestation level, and the collected lice were used for determining the in-vitro killing efficacy of selected traditionally used medicinal plants. We didn't use sheep for in vivo or any other test; rather, lice were collected. The sheep owners were informed about the purpose of the study, risks, and benefits in accordance with the level of their understanding in order to provide complete information, including the duration of the study and lice sample collection to be conducted from the sheep.

Availability of Data

The remaining plant powder and extract used during the study are kept at the College of Agriculture and Environmental Sciences Herbarium, Haramaya University, Ethiopia. All supporting datasets for this study are available upon request from the author.

Conflict of Interests

The authors declare that they have no conflicts of interest.

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Authors' Contributions

Sisay Alemu designed and coordinated the overall study. Sisay Alemu, Adugna Chalchisa, and Jelalu Kemal conducted the experiments. Sisay Alemu, Jelalu Kemal, Yehualashet Bayu, and Adugna Chalchisa participated in data collection, data analysis, interpretation of the results, and writing up of the manuscript. All the authors approved the final manuscript for submission.

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