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IN-VITRO LOUSCIDAL AND ACARICIDAL ACTIVITIES OF ALKALOID OF *CALPURNIA AUREA* EXTRACTS AGAINST *LINOGNATHUS OVILLUS* AND *AMBLYOMMA VARIEGATUM*

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ABSTRACT: The study was designed to evaluate the louscidal, and acaricidal activities of alkaloids of *Calpurnia aurea* leaves extracts. Alkaloid of *C. area* leaves extract at concentrations of 200, 100, 50, 25, 12.5 and 6.25 mg/ml were used for *in-vitro* adult immersion test of ticks and lice, which they were monitored for their mortality rates for 24 h. The activities of test substances were evaluated against *Amblyomma variegatum* and *Linognathus ovillus*, and compared with diazinon 60 EC. After 24 h post exposure, two higher concentrations of 200 and 100 mg/ml of the alkaloid extract caused 100 ± 0.5% and 100 ± 0.6% lice mortality, and 100 ± 0.33 and 93.3 ± 0.33 tick mortality respectively. The alkaloid extract showed an insignificant difference in its acaricidal and louscidal activity when compared to the Diazinon 60EC at the same concentration (P>0.05). LC_{50} and LC_{90} values (with 95% confidence limits) of the alkaloid of *C. aurea* for lice and tick were estimated 9.08 mg/ml (6.21-13.47), 17.65 mg/ml (11.71-22.49) and mg/ml 16.69 (11.77, 26.64), 31.69 mg/ml (21.25-50.72), respectively. Dose-response data of C. aurea alkaloid extract on *L. ovillus* and *A. variegatum* indicated the gradual increase in the mortality pattern with slopes of 3.1188, and 3.2321, and R^2 values of 0.9702 and 0.9882 suggesting that 97.02, 98.82% data were correlated with log concentration, respectively. The results obtained in this study indicate that the alkaloid extract of *C. aurea* has promising louscidal and acaricidal activities, lending support for further investigation of the plants to isolate the active components.

INTRODUCTION: Plants have played a central part in combating many ailments in human and livestock in many indigenous communities, including Africa [1]. Traditional healers, and particularly medicinal plant herbalists, in Africa, have a detailed knowledge-base of traditional medicine [2], which is transferred orally from one generation to the next through professional healers, knowledgeable elders and ordinary people [3]. Different parts of plants have been used to treat ectoparasites both in animals and man.

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These are roots, barks, leaves and seeds [4, 5]. *Calpurnia aurea* is widely distributed in Ethiopia. *Calpurnia aurea* leaves and powdered roots are used to treat stomach complaints, headache, eye diseases, scabies and skin infection caused by ticks and as an insecticide as well [6]. In Southern, Ethiopia peoples soak leaves of *C. aurea* in cold water to treat louse infestations (pediculosis) in humans and calves. In Western Ethiopia, the juice of crushed leaves and bark is used for tick control [7].

The identification of novel active plant-derived natural compounds could increase the number of available chemotherapeutic agents, thereby reducing the frequency of development of resistance and providing alternative drugs with greater acceptance, especially regarding environmental safety [8]. These shortcomings have prompted the search for alternative ectoparasites

control methods that are cheap and environmentally friendly like plant extracts [9]. Because of all the above background, use of botanical acaricides against highly pathogenic and economically important ectoparasites like ticks, mites and lice is extremely important. These problems have led to research efforts to discover new effective compounds.

Therefore, the objectives of the present study were:

- To evaluate *in-vitro* louscidal and acaricidal activities of the alkaloid of *Calpurnia aurea* leaves extract against *Linognathus ovillus* and *Ambylomma variegatum*;
- Compare acaricidal and louscidal activities of the isolated alkaloid extract obtained against *Linognathus ovillus and Amblyomma variegatum*.

MATERIALS AND METHODS:

Study Area: The laboratory experiment was conducted at Addis Ababa University College of Veterinary Medicine and Agriculture (CVMA), Parasitology laboratory at Bishoftu for louscidal activity test and Wollega University School of Veterinary Medicine, parasitology laboratory where acaricidal activity using in vitro adult immersion test was carried out.

Study Design: Experimental study in which the required some unsexed adult tick and lice were assigned to treatment and control group with replication were done. *In-vitro* acaricidal and louscidal efficacy of the extract of study plant on study parasite were evaluated.

Study Materials:

***Study Parasites*: Adult Sheep Lice:** *Linognathus ovillus* were collected from naturally infested sheep bought from the Ada'a district of East Shoa zone. Coat brushing technique was used for collection of lice from sheep. The parasites were maintained in plastic cups into which water soaked cotton are placed to increase the humidity of the air found in the cups. The cups were covered by gauze to allow the free circulation of air into the cups, and then the parasites were transported to CVMA, Parasitology laboratory. Identification of the parasites was conducted under a stereoscopic microscope according to the descriptions of Wall and Shearer [10]. Only, adult lice were used in these experiments.

Adult Ticks: *Amblyomma variegatum* was collected from cattle for the *in-vitro* acaricidal efficacy test from cattle brought to Diga Veterinary Clinic at Diga district of East Wollega zone. Ticks were collected from animals using forceps at main body sites namely: dewlap, brisket, belly and back, udder or scrotum, anogenital, and tail. Adult ticks collected from each of the main body sites were maintained in universal bottles separately and then transported to the Parasitology Laboratory of School of Veterinary Medicine, Wollega University for identification and *in-vitro* efficacy test.

Identification and recording of tick samples took place within a few h of collection. Ticks were identified using stereomicroscope following the standard identification procedures described by [11].

Study Plant: The plants to be evaluated were selected and harvested from field according to literature and their usage in ethnoveterinary medicine in the country. *Calpurnia aurea* leaves were further investigated based on the results of the efficacy of the crude extracts carried out previously [12]. Plant materials were collected from Wayu Tuka district, East Wollega zone of the Oromia region, located 331 km, West of Addis Ababa. This district is situated at an altitude between 1300 and 3140 meters above sea level. The annual temperature ranges from 12 °C to 32 °C, and the average annual rainfall varies between 1250-1850 mm [13]. The authenticity of the plant materials was confirmed by botanists at Aklilu Lemma Institute of Pathobiology (ALIPB), Addis Ababa, Ethiopia, where voucher specimens were deposited (collection numbers MA/001/06).

The Plant materials were dried in the shade, at ambient temperature, pulverized, and milled to powder mechanically. The powdered materials were separately stored in the dark tightly closed glass bottles at the Department of Pharmaceutical Chemistry and Pharmacognosy, School of Pharmacy, Addis Ababa University, for extraction. To reduce possible contamination, especially by fungi, latex gloves were worn when leaves were collected.

The Extracts were concentrated on a rotary evaporator under a reduced pressure of 22-26 mm Hg at 45 °C and the residues obtained were

weighed and stored at 4 °C [14]. During the preliminary screening, the adult tick and lice were used for bioassay test, and experiments were conducted for 24 h at room temperature (37°C ± 2°C).

Plant Extraction:

Extraction and Isolation of Alkaloids from *C. aurea*: Dried powder leaves of *C. aurea* (500 g) were defatted with petroleum ether (2000 ml) by maceration and filtered. The marc was further macerated with 80% methanol for 72 h. Extraction was repeated 3x and filtered. The combined filtrate was dried under reduced pressure using Rotavapor to obtain a greenish semi-sold hydroalcoholic extract. This extract was taken in 2% (100 ml) of HCl to obtain an acidic solution (pH ~ 2), which was partitioned with an equal volume of chloroform twice. The aqueous layer was basified with 10% ammonium hydroxide until a solution with a pH of 8-9 was obtained. The basic solution was extracted with an equal volume of chloroform 3x. The organic layers were combined and evaporated under reduced pressure to yield a reddish brown semi-solid [15].

Analytical Thin Layer Chromatography (TLC): Analytical TLC procedures utilized adsorption chromatography and were performed on silica gel 60 F_{254} precoated plates (0.2 mm) (E. Merck Darmstadt). The solvent system used for the alkaloids of *C. aurea* was a mixture of chloroform, methanol and 10% NH_4OH in a ratio of 90:9:1 (v/v). After air drying, the developed chromatograms were viewed under UV light. The alkaloids quenched UV light of short wavelength (wavelength 254 nm) and appeared as dark or brown bands on the plates. Following this, the alkaloids were visualized by spraying with Dragendorff's reagent made up as follows: 0.8 g of bismuth subnitrate in 50 ml of 20% v/v glacial acetic acid and 20 g of potassium iodide in 50 ml of distilled water were prepared separately and 5 ml of each of the solutions mixed with 90 ml of 22.2% v/v glacial acetic acid before use. The reagent gave orange-red color with all the alkaloids investigated [6].

***In-vitro* Louscidal and Acaricidal Efficacy Tests:** For the louscidal and acaricidal efficacy tests, the FAO modified protocol for adult immersion test was followed [17]. The test substance was diluted in distilled water and 2% DMSO at the concentrations required for the bioassays. The test extracts were dissolved in distilled water, and six concentrations were prepared arithmetically *viz*. 200, 100, 50, 25, 12.5 and 6.25 mg/ml were prepared by serial dilution. The *in-vitro* tests were carried within 1hr after lice collection [18].

Ten active lice and tick in three replications were placed in petri dishes, and 2 ml of each concentration was directly added to the three replicate Petri dishes and incubated at 27-28°C and 75-80% relative humidity for 24 h [19]. The tests were carried out on ten unsexed lice, and ticks per replication were carried out [20]. These three replicates were treated with distilled water and 2% DMSO, as negative and using diazinon 60 EC as positive controls [21].

The test solutions, positive (diazinon 60 EC) and negative (distilled water and 2% DMSO) controls were removed just after one and two minute contact time, using Whatmann no. 1 filter paper for lice and ticks respectively. The lice and tick in each Petri dish were closely observed for death under a stereomicroscope at 30 min, 1 h, 2 h, 3 h, 6 h, 12 h and 24 h time intervals [22].

The criteria used for determination of death of lice and ticks were extremely strict. If any signs of life such as movement of antennae, gut cells or minimal legs movements were observed with stimulation by needle, the lice are categorized as alive. The criteria for death of ticks were determined by observing any minor signs of life such as minimal legs movement and phalangial reflexes with stimulation by forceps, categorize the parasites as alive. The lice and ticks were judged as dead if there are no signs of movement at all [21]. The percent mortality rate of lice was calculated as per Abbot's formula cited by Krishnaveni and Venkatalakshmi (2014).

Mortality in the petri dishes treated with test substance was corrected to take account of control mortality using Abbott's correction. Classification of Louscidal and acaricidal effects are followed as previously used in *Melophagus ovinus* by [23;] as strong, mortality >80%; moderate, mortality 80-60%; weak, mortality 60-40%; little or no activity, mortality <40%.

Data Analysis: Collected raw data were stored in Microsoft Excel database system used for data management. SAS (r) Proprietary Software Version 9.00 (TS M0) was used for data analysis. Results of the study were expressed as a mean of mortality percentage ± standard error (Mean ± SE).

Statistical significance was determined by one-way analysis of variance (ANOVA) with multiple comparison tests (Post Hoc/Tukey's test/HSD) to compare parameter within and between groups. The LC_{50} and LC_{90} value of the extract were determined to apply regression equation analysis to the probit transformed data of mortality using SPSS windows version 20. The P values <0.05 were regarded as significant.

RESULTS:

TLC of the Alkaloid Extract of *Calpurnia aurea*: Examination of the alkaloid extract of *C. aurea* by analytical TLC using a mixture of chloroform, methanol and 10% NH_4OH indicated the presence of at least three alkaloidal components **Fig. 1**. Although only three Dragendorff's positive bands were observed on the chromatogram, it is possible that the number of alkaloids could be much more if other solvent systems are used.

FIG. 1: TLC CHROMATOGRAM OF THE ALKALOID EXTRACT OF *CALPURNIA AUREA*
(Adsorbent: Silica gel 60 F_{254} precoated plates (0.2 mm); Solvent system: Chloroform: Methanol: 10% NH_4OH (90:9:1); Visualization: Dragendorff's spray reagent).

***In-vitro* Louscidal Activity of Alkaloid Extract of *Calpurnia aurea*:** The alkaloid extracts from the leaf of *C. aurea* was tested for its louscidal activity against *Linognathus ovillus.* Percentage mortalities for the lice treated with the different concentrations of the *alkaloid* extract are shown in **Table 1**. The results showed that extract at concentrations of 200 mg/ml induced significantly (P<0.05) high levels of lice mortality compared to the reference drug diazinon at 30 min respectively. After 1hr exposure no significant difference in all concentration and the reference drug diazinon (P>0.05).

TABLE 1: LOUSCIDAL ACTIVITY OF DIFFERENT CONCENTRATION OF ALKALOID EXTRACT OF *CALPURNIA AUREA* ON *LINOGNATHUS OVILLUS* AT DIFFERENT TIMES OF EXPOSURE

Dose (mg/ml)	Mean mortality rate (%) ± SE						
	30 min	1 h	2 h	3 h	6 h	12 h	24 h
200	20 ± 0.5^{aa}	33.3 ± 0.3^{a}	46.6 ± 0.3^{ba}	60 ± 0.6^{ba}	70 ± 0.5^{a}	86.6 ± 0.8^{ba}	100 ± 0.6^{ba}c
100	13.3 ± 0.3^{ba}	20 ± 0.3^{a}	30 ± 0.5^{ba}	40 ± 0.3^{ba}	63.3 ± 0.3^{a}	86.6 ± 0.3^{a}	100 ± 0.5^{bac}
50	6.6 ± 0.3^{ba}	10 ± 0.3^{a}	16.6 ± 0.3^{ba}	26.6 ± 0.3^{a}	53.3 ± 0.3^{a}	63.3 ± 0.3^{ba}	86.6 ± 0.3^{ba}
25	3.3 ± 0.3^{b}	6.6 ± 0.3^{a}	20 ± 0.3^{a}	26.6 ± 0.0^{ba}	40 ± 0.3^{a}	50 ± 0.5^{ba}	70 ± 0.3^{ba}
12.5	3.3 ± 0.3^{b}	6.6 ± 0.3^{a}	13.3 ± 0.3^{ba}	23.3 ± 0.0^{ba}	30 ± 0.5^{a}	40 ± 0.0^{ba}	53.3 ± 0.5^{bac}
6.25	0 ± 0.0^{b}	3.3 ± 0.3^{a}	10 ± 0.3^{ba}	13.3 ± 0.3^{ba}	23.3 ± 0.5^{a}	$36.6 \pm 0.3b^{a}$	43.3 ± 0.3^{bc}
2% DMSO	0 ± 0.0^{b}	0 ± 0.0^{a}	0 ± 0.0^{b}	0 ± 0.0^{b}	0 ± 0.3^{a}	0 ± 0.0^{b}	0 ± 0.0^{c}
Diazinon (0.06%)	3.3 ± 0.33^{b}	10 ± 0.3^{a}	20 ± 0.0^{ba}	23.3 ± 0.3^{ba}	36.6 ± 0.3^{a}	66.6 ± 0.5^{a}	96.6 ± 0.5^{a}

Values are expressed as the mean of mortality % ± SE. Mortality % values with different superscripts within each column are significantly different (P<0.05).

The alkaloid extract at 200 and 100 mg/ml concentration after 6hrs of exposure showed the higher louscidal activity of 63.3% and 70% efficacy respectively. Besides, more than 50% of lice mortality was observed as early as 3 and 6 h after applying the alkaloid extract at the concentrations of 200 and 100 mg/ml, respectively. No mortality of Lice was found in the control group (treated with 2% DMSO). There were no statistically significant difference (P>0.05) in the louscidal activity among the 100 and 200 mg/ml concentrations after 24 h of exposure when compared to the reference drug (0.06% diazinon).

***In-vitro* Acaricidal Activity of Alkaloid Extract of *Calpurnia aurea* on *Amblyomma variegatum*:** *In-vitro* acaricidal activity of the Alkaloid extracts of *C. aurea* leaves was tested for its acaricidal activity against *A. variegatum*. Percentage mortalities for the ticks treated with the different concentrations of the alkaloid extract are shown in **Table 2**.

The results showed that the alkaloid at 200 and 100 mg/ml concentrations induced high levels of tick mortality as compared to other concentration resulting in killing 96.6%, 76.6% of tick within 12 h and 100%, 93.3% within 24 h **Table 2** respectively. The mortality of tick ranged from 46.6 ± 0.00 to 100.00 ± 0.33 at 24 h at 6.25 to 200 mg/ml concentrations, respectively. Also, more than 50% of tick mortality was observed as early as 6 h upon exposure to the alkaloid extract at concentrations of 200, 100 and 50 mg/ml, respectively. When mortality was compared to the positive control, there were no statistically significant differences between the two higher concentrations (P>0.05) after 6 h time exposure. When *A. variegatum* treated with all concentration of *C. aurea* alkaloid extract was compared to the negative controls (2% DMSO), there was a statistically significant difference (P<0.05).

TABLE 2: ACARICIDAL ACTIVITY OF DIFFERENT CONCENTRATIONS OF THE ALKALOID EXTRACT OF *CALPURNIA AUREA* ON *AMBLYOMMA VARIEGATUM* AT DIFFERENT TIMES OF EXPOSURE

Dose (mg/ml)	Mean mortality rate (%) ± SE						
	30 min	1 h	2 h	3 h	6 h	12 h	24 h
200	13.3 ± 0.33^{a}	20 ± 0.33^{ba}	36.6 ± 0.33^{a}	53.3 ± 0.33^{a}	73.3 ± 0.58^{a}	96.6 ± 0.33^{a}	100 ± 0.33^{ba}
100	6.6 ± 0.33^{a}	20 ± 0.33^{a}	33.3 ± 0.67^{a}	46.6 ± 0.33^{a}	60 ± 0.67^{a}	76.6 ± 0.33^{ba}	93.3 ± 0.33^{a}
50	3.3 ± 0.33^{a}	10 ± 0.33^{ba}	23.3 ± 0.33^{a}	33.3 ± 0.33^{a}	53.3 ± 0.33^{a}	66.6 ± 0.33^{ba}	83.3 ± 0.33^{a}
25	3.3 ± 0.33^{a}	10 ± 0.33^{ba}	13.3 ± 0.33^{a}	26.6 ± 0.33^{a}	43.3 ± 0.33^{a}	56.6 ± 0.33^{ba}	73.3 ± 0.33^{a}
12.5	0± 0.00^{a}	6.6 ± 0.33^{ba}	13.3 ± 0.33^{a}	23.3 ± 0.58^{a}	33.3 ± 0.58^{a}	46.6 ± 0.33^{ba}	63.3 ± 0.33^{a}
6.25	3.3 ± 0.33^{a}	3.3 ± 0.33^{ba}	10 ± 0.33^{a}	13.3 ± 0.33^{a}	26.6 ± 0.58^{a}	36.6 ± 0.58^{ba}	46.6 ± 0.00^{ba}
Diazinon 0.1%	6.6 ± 0.33^{a}	20 ± 0.33^{ba}	23.3 ± 0.58^{a}	30 ± 0.58^{a}	60 ± 0.58^{a}	83.3 ± 0.33^{a}	96.6 ± 0.33^{ba}
DMSO (2%)	0 ± 0.00^{a}	0 ± 0.00^{b}	0 ± 0.00^{a}	00 ± 0.00^{a}	0 ± 0.00^{a}	1 ± 0.33^{b}	3.3 ± 33^{b}

*Values are expressed as the mean of mortality % ± SE. Mortality % values with different superscripts within each column are significantly different (P<0.05).

Different concentrations of alkaloid of *C. aurea* extract depending on the time on *L. ovillus* and *A. variegatum* were tested and regression equations were showed in **Fig. 2**. Dose-response data of alkaloid *C. aurea* indicated the gradual increase in the mortality pattern with a slope of 3.1188 and the R^2 value of 0.9702 suggesting that 97.02% data were correlated with log concentration.

Acaricidal and Louscidal Activity Comparison between *Calpurnia aurea* Alkaloid: After 24 h post exposure, 200 and 100 mg/ml doses of the alkaloid extract caused lice mortality of 100 ± 0.6% 100 ± 0.5%, whereas the percentage mortality levels against ticks were 100 ± 0.33 and 93.3 ± 0.33, respectively. After 24 h, the LC_{50} and LC_{90} values (with 95% confidence limits) of the alkaloid extract for lice and ticks were estimated 9.08 mg/ml (6.21-13.47), 17.65 mg/ml (11.71-22.49) and 16.69 mg/ml (11.77, 26.64) and 31.69 mg/ml (21.25-50.72), respectively **Table 3**. Based on the LC_{50} and LC_{90} values, the alkaloid extract was found to be more effective in killing of lice than tick **Table 3**.

TABLE 3: LC_{50} AND LC_{90} WITH 95% CI OF ALKALOID EXTRACT OF *CALPURNIA AUREA* OBTAINED BY AIT AGAINST *AMBLYOMMA VARIEGATUM* AND *LINOGNATHUS OVILLUS* AFTER 24 h TIME EXPOSURE

Plant Extracts	Test parasite	Regression equation	R^2	LC_{50} (mg/ml) (95 % CI)	LC_{90} (mg/ml (95 % CI)
Alkaloid	lice	y = 3.1188x - 2.9885	0.9702	9.08 (6.21-13.47)	17.65 (11.71-22.49)
	tick	y = 3.2321x - 3.9512	0.9882	16.69 (11.77-26.64)	31.69 (21.25-50.72)

*Tick = *Amblyomma variegatum*, lice = *Linognathus ovillus*

DISCUSSION: Extraction of alkaloids from the 80% methanolic extract of *C. aurea* leaves yielded 0.5% (w/w) of alkaloids. The previous report by [6] indicated that the hydroalcoholic extract of *C. aurea* leaves contains 1.65% (w/w) alkaloids. The variability in the yield of alkaloids could be attributed to the time of collection which is among the main factors that affect alkaloid contents in plants. Although the same authors isolated 13 quinolizidine alkaloids, only two of the alkaloids constituted 90% of the alkaloidal content of the plant. In the present study, TLC examination of the crude alkaloid extract of *C. aurea* leaves furnished three major bands that may contain more than three alkaloids.

Investigation of the alkaloid extract of *C. aurea* leaves for its louscidal and acaricidal effects against *L. ovillus* and *A. variegatum*, respectively, revealed that it possesses potent activities against both ectoparasites. At higher doses of 200, 100 and 50 mg/ml, the extract showed comparable louscidal and acaricidal activities to the standard drug diazinon. To the best of our knowledge, no report exists in the literature concerning the louscidal activity of the alkaloids of *C. aurea* against *L. ovillus*, although [24] has documented their insecticidal properties.

In a previous study, it has been reported that that the insecticidal properties of some plant extracts could be due to the involvement of anticholinergic alkaloids such as scopolamine, hyoscyamine, meteloidine, and atropine [25]. As *tropane* alkaloids have not been isolated from *C. aurea*, *quinolizidine* alkaloids could be regarded as another class of alkaloids that possess insecticidal properties.

Comparative studies on the acaricidal and louscidal effects of the alkaloids of *C. aurea* against *L. ovillus* and *A. variegatum* indicated that the alkaloids are more active on lice than ticks. Moreover, from the calculated LC_{90} values after 24h time exposure **Table 3**, it is seen that the effect of the alkaloids against lice was in a dose-dependent manner, *i.e.* percentage mortality increased with an increase in the dose of the extract. This is in line [26], who reported that difference between a medicinal and a toxic effect of many alkaloids (or any drug) is often a matter of dosage.

Acaricidal and louscidal activities of the alkaloid extract of *C. aurea* against *L. ovillus* and *A. variegatum* illustrate those correlations exist between the popular ancestral perception and genuine antiparasitic activities. Moreover, it lends support to further studies aimed at the isolation and identification of alkaloid(s) with better therapeutic value. Although the alkaloid extracts of *C. aurea* were found to be lethal to *L. ovillus* and *A. variegatum*, they appear to be more active against *L. ovillus* than *A. variegatum*. This might be attributed to a thickness of the integument in ticks as compared to lice, which may interfere with the absorption of the active ingredients present in the plant extracts. However, the mechanism(s) by which the plant extracts exert their action must be studied in order to know their actual mode of action.

CONCLUSION AND RECOMMENDATIONS: The present study has demonstrated the *in-vitro* acaricidal and louscidal activities of the alkaloids of *Calpurnia aurea* against *Linognathus ovillus* and *Amblyomma variegatum*. The current study showed that the alkaloids of *Calpurnia aurea* possess strong activity which was comparable to that of the reference drug diazinon. There is a high possibility that these plant extracts provide effective, eco-friendly herbal formulations for the control of lice and tick infestation on animals.

In line with above-concluding remarks, the following recommendations are forwarded:

- Phytochemical investigations of the active extracts must be carried out to isolate and elucidate the structure(s) of the active constituents.
- Acute and chronic toxicity studies should be done to assess the safety of the extracts under field conditions.
- Further, louscidal and acaricidal activity tests on other ectoparasites need to be done to know if the plants have a broad spectrum of activity.

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AUTHORS' CONTRIBUTIONS: MA: Data collection, interpretation of the results, and drafting the manuscript. YH, GT, KA: Conception of the research idea, designing Data collection, interpretation of the results and drafting the manuscript with. All authors read and approved the final manuscript.

REFERENCES:

1. Busman WR, Swartzinsky P, Worede A and Evangelista P: Plant use in Odo-Bulu and Demaro, Bale region Ethiopia. Journal of Ethnobiology and Ethnomedicine 2011; 7: 28.
2. Moshi MJ, Otieno DF, Mbabazi PK and Weisheit A: The Ethnomedicine of the Haya people of Bugabo ward, Kagera Region, North western Tanzania. Journal of Ethnobiology and Ethnomedicine 2009; 5: 24-43.
3. Giday M, Teklehymanot T, Abebe A and Yalemtsehay M: Medicinal plants of the Shinasha, Agew-awe and Amhara peoples in Northwest Ethiopia. Journal of Ethnopharmacology 2007; 110(3): 516-25.
4. Bekele D, Asfaw Z, Petro B and Tekie H: Ethnobotanical study of plants used for protection against insect bite and for the treatment of livestock health problems in rural areas of Akaki district, Eastern Shewa, Ethiopia. Journal of Herbal Medicine 2012; 1: 12-24, 26.
5. Teklay A, Balcha A and Mirutse G: Ethnobotanical study of medicinal plants used in Kilte Awulaelo District, Tigray Region of Ethiopia. Journal of Ethnobiology and Ethnomedicine 2013; 9: 1-23.
6. Asres K, Bucar F, Kartnig T, Witvrouw M, Pannecouque C and De-Clercq E: Antiviral activity against human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) of ethnobotanically selected Ethiopian medicinal plants. Phytotherapy Research 2001; 15: 62-69.
7. Alawa CBI, Adamu AM, Gefu JO, Ajanusi OJ, Abdu PA, Chiezey NP, Alawa JN and Bowman DD: *In-vitro* screening of two Nigeria medicinal plants (*Vernonia amygdalina* and *Annona senegalensis*) for anthelmintic activity. Veterinary Parasitology 2003; 113: 73-81.
8. Zorloni A, Penzhorn BL and Eloff JN: Extracts of *Calpurnia aurea* leaves from southern Ethiopia attract and immobilize or kill ticks. Veterinary Parasitology 2010; 168: 160-164.
9. Wall R and Shearer D: Veterinary External Parasites: Biology, Pathology and Control, Blackwell Science LTD, 2nd edition. Blackwell Science, UK 2001; 23-54.
10. Walker AR, Bouattour A, Camicas JL, Estrada-Peña A, Horak IG, Latif AA, Pegram KG and Preston PM: Ticks of domestic animals in Africa: A guide to Identification of Species. Bioscience Reports 2003; 221.
11. Sisay A: Study on *in-vitro* louscidal and acaricidal properties of *Calpurnia aurea*, *Otostegia integrifolia*, *Nicotiana tabaccum* and *Jatropha curcas* against *Bovicola ovis* and *Amblyomma variegatum*. MSc thesis, Addis ababa University, FVM 2015.
12. NMSA: National Meteorology Service Agency. East Wollega Branch, Nekemte, Ethiopia 2014.
13. Eloff JN, Famakin JO and Katerere DRP: Combretum woodii (Combretaceae) leaf extracts have high activity against Gram-negative and Gram-positive bacteria. African Journal of Biotechnology 2005; 4(10): 1167-1171.
14. Yubin JI, Miao YU, Wang B and Zhang Y: The extraction, separation and purification of alkaloids in the natural medicine. J Chem Pharm Res 2014; 6(1): 338-345.
15. Sandam N and Su P: TLC-bio autography guided screening of the methanolic extract of Ricinus communis. International Journal of pharma and biosciences 2015; 6(1): 427- 432.
16. FAO: Resistance management and integrated parasite control in ruminants: Guidelines. http://www.fao.org/ag/aga.html 2004.
17. Heukelbach J, Oliveira FAS and Speare R: A new shampoo based on Neem (*Azadirachta indica*) is highly effective against head lice *in-vitro*. Journal Parasitology Research 2006; 99: 353-356.
18. Sanis J, Reghu R, Sunil A, Ramankutty A, Kumar KG, Suresh NN, Amithamol KK, Amitabh BA, Kumar SR and Srikanta G: *Jatropha curcas* (Linn) leaf extract -a possible alternative for population control of Rhipicephalus (Boophilus) annulatus. Asian Pacific Journal of Tropical Disease 2012; 5: 225-229.
19. Nchu F, Magano SR and Eloff JN: *In-vitro* investigation of the toxic effects of extracts of *Allium sativum* bulbs on adults of *Hyalomma marginatum* rufipes and *Rhipicephalus pulchellus*. Journal of the South African Veterinary Association 2005; 76: 99103.
20. Jadhav V, Kore A and Kadam VJ: *In-vitro* pediculicidal activity of *Hedychium spicatum* essential oil. Fitoterapia 2007; 78: 470-473.
21. Nanaa PB, Manianiaa NK, Marangab RO, Kutimab HL, Bogab HI, Nchuc F and Eloff D: Attraction response of adult *Rhipicephalus appendiculatus* and *Rhipicephalus pulchellus* (Acari: Ixodidae) ticks to extracts from *Calpurnia aurea* (Fabaceae). Veterinary Parasitology 2010; 174: 124-130.
22. Gemeda N, Mokonnen W, Lemma H, Tadele A, Urga K, Addis G and Gebre S: Insecticidal activity of some traditionally used ethiopian medicinal plants against Sheep Ked *Melophagus ovinus*. Journal Parasitology Research 2014; 3-4: 7.
23. Silva-Aguayo G: Botanical insecticides. Radcliffe's IPM World Textbook. The University of Minnesota. Retrieved January 8, 2016, from www.Radliffes/ipm/network.Htm. 2006.
24. Berkov S, Doncheva T and Philipov S: Comparative study of the alkaloids in tribe Datureae and their chemo-systematic significance. Biochemical Systematics and Ecology 2006; 34(6): 478-488.
25. Levetin E and McMahon TK: Plants and society. McGraw-Hill, Dubuque, Iowa, Edition 3rd, 2003.

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