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Research Article

Phytochemical Screening and Invitroacaricidal Activity of three Herbal Extracts aginst Cattle Tick Boophilus Decoloratus

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Abstract

Boophilus decoloratus tick is an economically important ecto parasite of the cattle and that generates major problem for livestock producers. Commonly, these ecto parasite are controlled by commercial acaricides produced by manufacturers however, increase in resistance, environmental toxicity, scarcity and high cost led to the evaluation of other alternative tick controlling option. This expermintalstudy was designed to determine the acaricidal efficacy of *Daturastramonium*, *Nicotianaglauca* and *Azadirachtaindica* herbal extracts against the common cattle tick *Boophilusdecolratous*. Qualitative phytochemical screening was used to detect secondary metabolites contained in the selected herbs. The percentage of adult mortality and percentage inhibition of oviposition were studied at different experimental concentrations of 25, 50, 75 and 100mg/ml to determine the efficacy of leaf extracts. 3% of Dimethyl sulfoxide was also used as a negative control. The study determined the presence of secondary metabolites such asalkaloids, tannins, glycosides in the herbal leaf extracts, which are able to cause neuro toxicity in the tick. Inhibition of oviposition at the highest concentration of *Daturastramonium*, *Nicotianaglauca* and *Azadirachtaindica* of the treated ticks were 78.68, 86.84 and 52.63%, respectively. At the highest concentration, the adult tick mortality was 90, 100 and 80% for *Daturastramonium*, *Nicotianaglauca* and *Azadirachtaindica*, respectively. The results of the current study pointed the potential acaricidal effect of selected medicinal plants with varying potency. Therefore, further study should be done in vitro and in vivo assays to use studied plants as acaricides.

Key words: Azadirachtaindica; Boophilusdecoloratus; Daturastramonium; Nicotianglauca.

Abbreviations

A. Azadirachta indica
B. Boophilus decoloratus
D. Daturas tramonium
DMSO-Dimethyl sulfoxide
N. Nicotiana glauca

Introduction

Among the major health concerns disturbing livestock the one is ecto-parasites, particularly tick infestation. Directly, ticks causes skin damage opening up wounds which make the animal susceptible to secondary infection. Indirectly and more significantly, ticks act as vectors of fatal diseases, like babesiosis, cowdrosis and theileriosis [8].

In common practice, these ecto parasites are controlled by commercial chemical acaricides. However, the use of commercial acaricides has been led to development of widespread resistance, environmental toxicity of chemicals, residuals in animal product and ever increasing cost of acaricides [5]. Medicinal plants are important for healing of human and animal diseases due to the existence of

certain specific substances, known as phytochemicals. Phytochemicals are not nutritious chemical compounds and are naturally found in medicinal plants which results defense mechanisms and protection against various diseases [11]. In Ethiopia, Medicinal plants and knowledge of their use provide a vital contribution to human and livestock health care needs throughout the country. Available literature clearly shows that the contribution of medicinal plant as a primary health care options in the country, where 70% of human and 90% of livestock population depend on traditional medicine [1].

So the present study detect the type of secondary metabolites present in crude ethanolic extracts of *Azadirachtaindica*, *Daturastramonium*, *Nicotianglauca* and its acaricidal effect of herbal extracts against the cattle tick B. decolratous.

Materials and Methods

Study Herbs and Collection

The herbs used in the present experimental study were *Daturastramonium*, *Nicotianaglauca* and *Azadirachtaindica*. The leaves were harvested from mekelle city districts.

Herbal Extraction

The collected plant materials were washed with tap water to remove any traces of soil and other unnecessary particles. Leaves were air dried completely under shade and ground with electric blender into fine powder, sieved and stored in clean stopper bottles until used for extraction. The powdered leaves were macerated using 97% Ethanol. Two hundred fifty grams of each experimental leave powders were weighted using an electronic weighing balance and mixed with 1250 ml of 97% Ethanol in each of the flasks and plugged tightly with plastic and sterile gauze. The coarsely powdered materials were kept in contact with selected solvent in the flasks for three days with frequent agitation manually four times per day for 10 minutes until soluble matter was dissolved. Then, the liquid part was separated from the herbal residues and filtered through a what man filter paper number 1 using an electrical suction pump. The beaker with liquid then put in a water bath at 42°C to evaporate the solvent. The weights of the dried crude extracts were determined and a yield percentage was calculated for each experimental herbs.

Phytochemical Screening

The crude extract of A .*indica*, N. *glaucaand* D.*stramoniumwere* subjected to phytochemical screening using the generally accepted laboratory techniques for qualitative determinations. Screening was carried out for crude extracts to identify the active chemical constituents [7].

Test for saponins

Frothing test: this test was performed by mixing 1gm of crude extract with 5ml of distilled water in a test tube and it was shaken vigorously for 15 minutes. The formation of stable foam that remains for 10-15 minutes indicated the presence of saponins.

Test for flavonoids

Shinoda test: this test was performed by mixing 0.1g of the extracts with few drops of 1%NaOH. An intense yellow color was produced in the extract which became colorless on addition of a few drops of diluted HCl acids indicates the presence of flavonoids.

Test for tannins

Ferric chloride test: About 0.5 g of the extract in 10 ml of water in a test tube in a beaker was boiled on hot plate stirrer for 5 minutes and then filtered with filter paper. Two drops of 0.1% ferric chloride solution was added and observed for brownish or blue black coloration indicating the presence of tannins.

Test for alkaloids

Mayer's test: This test was performed by mixing 1g of the extract with 2ml of HCl and heated on water bath for 5 minute and 3 drops of Mayer's reagent was added and checked for creamy white precipitate.

Test for phlobatannins

Hydrochloric acid (HCl) test: About 0.2 g of extracts were added to 2 ml of 1% HCl in test tube in beaker and the mixture was boiled on hot plate stirrer and cooled for 5 minutes. Deposition of a red precipitate was taken as evidence for the presence of phlobatannins.

Test for phenolic compounds

Ferric chloride test: In this test 0.2 g of extract was dissolved in 5ml of distilled water, and 2 ml of 5% ferric chloride solution was added. The formation of bluish green color indicated presence of phenolic compounds.

Test for Glycosides

Sulfuric acid (H_2SO_4) test: in this test 2ml of concentrated H_2SO_4 was added carefully to 1g of each crude extract in test tube and shaken gently. A reddish brown color was observed for all extracts. This indicated the presence of glyconeportion of the glycoside.

Preparation of Experimental Concentrations

The experimental concentrations used to measure efficacy of three experimental herbal to were 100mg/ml, 75mg/ml, 50mg/ml and 25mg/ml. 3% Dimethyl sulfoxide (DMSO) used as solvent and control group.

Experimental tick collection and identification

Adult male and engorged B. *decoloratusticks* were collected from intensive farms from Mekelle city. Cattle were restrained and the entire body surfaces of the animals were examined thoroughly and the ticks were collected from neck, shoulder, belly, udder, anus and legs. The ticks were transported to the veterinary parasitological laboratory and examined under stereomicroscope for identification according to the standard identification keys [16].

Adult Immersion Test (AIT)

The investigational procedure suggested by [2] and modified by (FAO) was used to carry out the experiment. The engorged female ticks collected from the field were washed thoroughly with tap water and kept for drying on filter paper. Ten viable ticks were put in group and weighed; an effort was made to obtain groups with similar weights. Three replicates of five different test tubes were made ready each containing the different crude extract concentrations (100, 75, 50 and 25mg/ml) and the control (3% DMSO). The ticks on each group were immersed into each experimental concentration for 5 minutes. The ticks were removed out of the extract solutions and gently dried on filter paper. Then these ticks were kept separately in petri dishes with filter paper at bottom. The petri dishes were covered with sterile gauze and put in a biochemical oxygen demand (BOD) incubator at 28±2°c with a relative humidity of 80% for 7 days. After 7 days of incubation, the ticks on each petri dishes were observed for egg laying and eggs produced by each group were weighed. Then the efficacy of the crude extracts was evaluated using percent inhibition of oviposition (IO%) and which was calculated by;

$$IO\% = \frac{MEC - MET}{MEC} \times 100,$$

Where, MEC and MET are mass of eggs laid by control ticks and treated ticks, respectively and IO (%) percent inhibition of oviposition.

Filter Paper Impregnation Method (FPIM)

The experimental procedure adopted by FAO [3] was used to investigate adult tick mortality. Whatman filter papers of the same diameter as that of the Petri-dishes were impregnated with one ml of each experimental concentration and control. Then these filter papers were placed in the petri dishes and ten adult ticks were put onto these filter papers of each petri dishes. A similar filter paper impregnated with the same strength of the extract was placed on top of the ticks. Three replications were done for each concentration. The petri dishes were then closed with sterile gauze. Tick mortality was then recorded after 24 hrs of exposure to crude extracts and the control. The criteria for death of ticks were determined by observing signs of the movement and by pricking it with needle. The ticks were judged as dead, if there are no signs of movements. Finally, the mortality of the adult tick was calculated by using the formula:

$$\text{Mortality (\%)} = \frac{\text{Dead tick count}}{\text{Total tick count}} \times 100$$

Data management and analysis

Data of the variables of interest such as concentrations of the experimental extracts, tick egg weight and mortality percentages were entered into Microsoft excel spread sheet and descriptive statistics of the collected data was calculated. Percent inhibition of oviposition and mortality percentage for each leaf extract was used to determine its effectiveness.

Results

Yield Percentages of the Herbal Extracts

Plants	***Weight of the fine powder***	***Amount of dried crud extract***	***Yield %***
D. *stramonium*	250g	33.2g	13.28%
N. glauca	250g	29.19g	11.68%
A. *indica*	250g	27.19g	10.87%

Table 1: Yield Percentage of Ethanolic Extracts of the Experimental Herbs.

Phytochemical Screening

The qualitative screening of crude extract of all selected plants showed the presence alkaloid.

Variable	***D. Stramonium***	***N. glauca***	***A. Indica***
Alkaloids	+	+	+
Tannins	_	+	+
Flavonoids	_	_	_
Saponins	+	_	_
phenolic compounds	_	+	+
Phlobatannins	_	_	_
Glycosides	_	+	+

Note: + is present, - is absent.

Table 2: Phytochemical Analysis of Ethanolic Extracts of the Experimental Herbs.

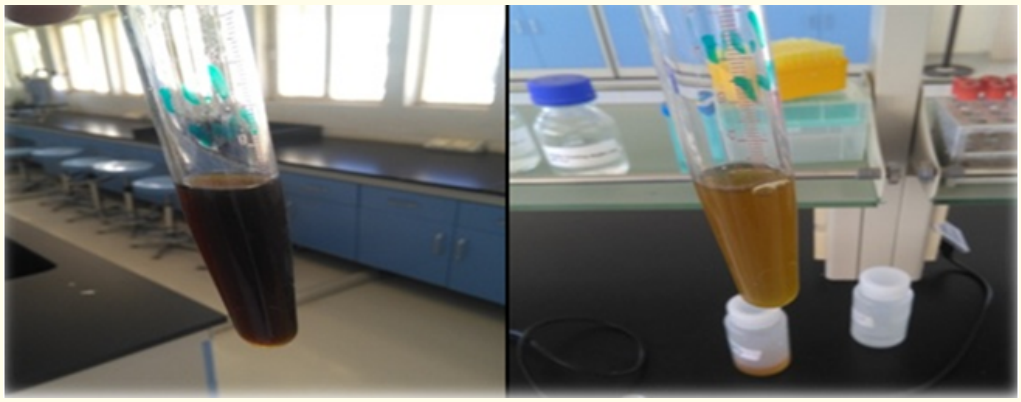

Figure 1: Tannins Positive and Flavonoids Negative Respectively during Phyto Chemical Analysis.

Percentage Inhibition of Oviposition

The percentage inhibition of oviposition of B. *decoloratus* at highest concentration is 78.6%, 86.84% 52.63% for D. *stramonium*, N. *glaucaand* A. *indicarespectively*.

Plant	***concentration mg/ml***	***N***	***M2***	***IO***
D.*stramonium*	Control	3	0.38	0.00
	100mg/ml	3	0.081	78.68%
	75mg/ml	3	0.094	75.26%
	50mg/ml	3	0.11	71.05%
	25mg/ml	3	0.13	65.78%
N. *glauca*	100mg/ml	3	0.05	86.84%
	75mg/ml	3	0.062	83.68%
	50mg/ml	3	0.069	81.84%
	25mg/ml	3	0.083	78.15%
A.indica	100mg/ml	3	0.18	52.63%
	75mg/ml	3	0.21	44.73%
	50mg/ml	3	0.22	42.10%
	25mg/ml	3	0.26	31.57%

N= Number of immersed engorged ticks; ***M2***= mean egg mass per replicate (g); ***IO***= percent inhibition of oviposition.

Table 3: The results of adult immersion test of ethanolic leaf extracts

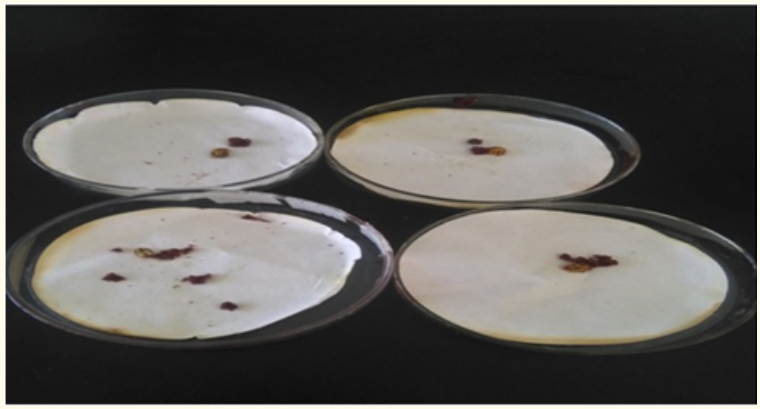

Figure 2: Egg mass lied by Engorged B. decolratous.

Percentages of adult tick mortality

Among the three plants N. *glaucashows* effective mortality to the adult tick then followed with D.*stramonium* and A. *indica* on different expirmental concentrations.

Plant	***concentration (mg/ml)***	***N***	***T***	***Mortality %***
D.*stramonium*	Control	10	24hr	0.00%
	100mg/ml	10	24hr	90%
	75mg/ml	10	24hr	80%
	50mg/ml	10	24hr	80%
	25mg/ml	10	24hr	60%
N. *glauca*	100mg/ml	10	24hr	100%
	75mg/ml	10	24hr	100%
	50mg/ml	10	24hr	100%
	25mg/ml	10	24hr	80%
A. *indica*	100mg/ml	10	24hr	80%
	75mg/ml	10	24hr	70%
	50mg/ml	10	24hr	60%
	25mg/ml	10	24hr	50%

***N*=**numbers of tick, ***T*=**time exposure.

Table 4: Percentages of Mortality of B. *Decolratous* tick by Experimental Ethanolic Extracts.

Discussion

The study showed the presence of biologically active compounds in the experimental ethanolic leaf extracts of N.*glauca*, D.*stramonium* and A. *indica*. Due to the existence of different bioactive compounds in leaf extracts, antibacterial, antifungal, antiparasitc and anti-inflammatory properties have been attributed to it [14]. In previous studies it was reported that phenolic compounds and Tannins were absent in ethanolic extract of N.glauca [17]. However, the result of this studies showed that both secondary metabolites were present in the ethanolic leaf extract. Also the pervious study by Prashanth *et al* [12] showed that Saponins and Flavonoids are present in ethanolic extracts of A. Indic.But the current finding of this study shows both bioactive compounds were found to be absent. The outcome of this studies and earlier research studies results were unlike so it might be due to the change in location and genetic variation due to cross pollination, so their genetic makeup were changed and that is why both studies shows different results. This study revealed the presence of secondary metabolite like alkaloids in all leaf extracts. Also it shows the presence of biologically active compounds such as tannins, phenolic compounds and Glycosides in selected plants except D. *Stramonium*. This phyto constituents' of medicinal plants are considered to be the chemical components that are so potent and are reported to cause mortality and inhabition of ovipostion of adult ticks [10].

The study by Ganeshalingam (2011) investigated toxic effect of the leaf extracts of Datura spices plants at various concentrations on grasshoppers and red ant. In this study, D. stramonium was evaluated against B. *decolrtustick* and shows inhibition of oviposition of engorged female ticks from 65.78% to78.68%, when tested at concentrations ranging from 25 to 100 mg/ml. The highest inhibition of ovipostione (78.68%) was observed at the higher concentration (100 mg/ml). The percent of adult tick mortality caused by the crude ethanolic extract of this herb is varied from 60% to 80% when tested at concentrations ranging from 25 to 100 mg/ml at exposure time of 24 hours. This results were slightly in agreement with the study made by Srikantaet al. [15] onethanolic extract of D. *stramoniumin* India, who reported 60% mortality of adult tick and 83 % percentage inhibition of oviposition at 100mg/ml.

The petroleum fractions of A. *indica* seed also evaluated in *vitro test* and found effective gainst B. microplus tick [6]. In the present study, the ethanolic leaf extract of A. *indica* were evaluated against B. *decolrtusand* imparted inhibition of oviposition ranging from 52.63% to 31.57% at different experimental concentrations. The percent mortality caused by the ethanolic extracts A. *indica* is varied from 50% to 80% at concentrations ranging from 25 to 100mg/ml. The highest mortality and inhibition of oviposition was observed at 100mg/ml. This finding is in agreement with Kalakumar *et al* [9] also assessed the effect of A. *indica* and have shown 60-75% efficacy against buffalo ticks but failed to inhibit oviposition in female ticks; but, the present finding, in contrary to this, reported up to 52.63% % inhibition in oviposition. Related results were reported also with Rahul *et al* [13] in which 65% mortality of adult Bopilus tick was recorded within 24 h of treatment with extract A. *indica* at 100mg/ml.

In this study, the leaf extracts of N. *glauca*, shows good acaricidial activity from the other selected herbal extracts. It shows the highest percentage of inhibition of oviposition and mortality of adult ticks at all concentration when compared to D. *Stramonium* and A. *indica*. At concentration of 100mg/ml, the leaf extract of N. *glaucaexhibited* 86. 84% of inhibition of oviposition and 100% of mortality rate in the present study is in contrast with Flávia finding [4]. 2014 in Brazil, who reported 22.64% of inhibition of oviposition and 46.46 mortality rates at the same concentration against B. *microplus*. The result of this study and previous research studies results were different so it might be due to the variation in phyto constituents of the plant from the place to the place. Further investigations are recommended in this regard.

Conclusion

The findings from this study determine that the crude ethanolic extracts of N.*glauca*, D.*stramonium* and A. *indica* contain secondary metabolites with in *vitroacaricidal* activities against adult B. *decolratoustick* and showed mortality and inhibition of oviposition effect especially N.*glauca* and D.*stramonium* showed high efficacy.

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