



Evaluation of Anthelmintic Activity of Tropical Tanniferous Plant Extracts Against *Haemonchus contortus*

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Mastewal Birhan ¹
Tilahun Gesses ¹
Ambaye Kenubih ¹
Haileyesus Dejene ²
Muluken Yayeh¹

¹Department of Veterinary Paraclinical Studies, College of Veterinary Medicine and Animal Sciences, University of Gondar, Gondar, Ethiopia; ²Department of Veterinary Epidemiology and Public Health, College of Veterinary Medicine and Animal Science, University of Gondar, Gondar, Ethiopia

Introduction: The present trial aimed to investigate invitro anthelmintic activities of selected tropical tanniferous plant extract on egg hatchability and larval development inhibition of *Haemonchus contortus* in sheep. In view of that, three tropical tanniferous plants *Rhus glutinosa*, *Syzygium guineense* and *Albizia gumifera*, were selected based on their relative high content of condensed tannins.

Methods: In this study, eggs were collected from artificially infected with *H. contortus*. Then the egg was directly subjected to invitro assay with these condensed tannin-enriched extracts using egg hatchability assay and inhibition of larval development assay.

Results: The result showed that extracts from all three tropical tanniferous plants demonstrated statistically significant ($P < 0.05$) dosedependent inhibition of both egg hatchability and larval development. According to IC_{50} and IC_{90} values, the condensed tannin-enriched extracts inhibiting egg hatching and larval development most potently were *Rhus glutinosa* followed in descending order of activity by *Syzygium guineense* and *Albizia gumifera*.

Discussion: The result of this study showed that these condensed tanninriched extracts were effective in inhibiting egg hatchability as well as larval development. Therefore, condensed tannin might be recommended as one of the options for the control of *H. contortus* in sheep.

Keywords: *Haemonchus contortus*, condensed tannin, anthelmintic, larval development assay, egg hatchability assay

Introduction

Small ruminant farming has a prominent role in the sustainability of rural communities around the world, as well as being socially, economically and politically highly significant at national and international levels, as with all livestock species. Thus, the factors that negatively affect livestock production infections with parasites in particular gastrointestinal nematodes continue to represent a serious challenge to the health, welfare, productivity and reproduction of grazing ruminants throughout the world.¹ Helminth parasites play an important role in small ruminant production leading to enormous economic losses through mortality, weight loss, reduced milk, meat and wool production.^{2,3} *Haemonchus contortus* is the most important of all gastrointestinal helminths that constrain the survival and productivity of sheep owned by poor rural farmers in the developing world.⁴

The economic importance of these parasitic infections in ruminants imposes the need for rigorous measures of control. Thus, these measures relied on the repeated

Correspondence: Mastewal Birhan
Email maste675@gmail.com

use of synthetic, chemical anthelmintic drugs in order to break the nematodes' life cycles by eliminating the worms in the hosts. However, this nearly exclusive reliance on synthetic molecules to control GI nematodes nowadays faces several limits. The first is the enhanced concern of consumers about the use of chemicals in farm animals, which might generate possible residues in food products or environmental consequences,⁵ the threat of anthelmintic resistance and high cost, especially to farmers of low income in developing countries, which have led to the notion that sustainable helminth control cannot be achieved with commercial anthelmintics alone. Therefore, other options, such as biological control, vaccines and traditional medicinal plants are being examined in different parts of the world.⁶

The Ethiopian custom of using medicinal plants for the treatment of intestinal parasites has existed for many generations.⁷ Some tannin-rich plants can have direct anthelmintic effects against the main nematode species of sheep and goats.^{8,9} Moreover, some authors have reported a relatively good effect of condensed tannins on worm burden of abomasum worms¹⁰ after the use of condensed tannins in ruminant diet. It has also reported that certain plants with high condensed tannin content are accepted by browsing sheep, making them possible candidate for nematode management.¹¹

Ethnoveterinary surveys conducted so far in Ethiopia indicate that several traditional healers use medicinal plants for the treatment of various animal health problems including the treatment of helminth infection.^{7,12–14} However, very few efforts have been made to scientifically screen and evaluate the anthelmintic effect of condensed tannins. Therefore, the aim of this study was to investigate the *in vitro* anthelmintic activities of selected condensed tannin plants on the egg and larval development of *Haemonchus contortus*.

Materials and Methods

Plant Collection and Extraction of Condensed Tannins

As described previously^{8,15} and based on traditional knowledge obtained from the study area, fresh leaves of *Rhus glutinosa*, *Syzygium guineense* and *Albizia gumifera* were harvested from their natural habitat in and around Bahirdar area including Blue Nile river basin (LATITUDE AND LONGITUDE), Amhara regional state, Ethiopia. Thereafter, the plant materials were dried in a well-aerated

dark room to avoid direct sun light. Then, 500 g of dried leaves were pulverized and placed in a mixer containing 3 L of 70% acetone in water containing ascorbic acid (1 g/L) to avoid oxidation. After that, the mixture was sonicated in a water bath for 20 min. The extract was obtained from the filtered material using a filter paper. Meanwhile, the acetone was evaporated from the extract at 45°C (in vacuo) using a roto-vapor (BUCHI, England). The aqueous solution was washed four times with 500 mL methylene chloride to remove chlorophyll and lipids. A separation funnel was used for discarding the methylene chloride fraction. The remaining fractions were lyophilized (Ningbo, China) and kept refrigerated at 4°C in airtight containers until their use for biochemical and biological assays.

Tannin Quantification by Acid Butanol Method

To assay soluble CTs, 0.5 mg of tannin-enriched extract was mixed with 2 mL of butanol-containing reagent (51.5% acetone/43% butanol/5% 12 N concentrated HCl/0.5% H₂O) and 67 µL of ferrous reagent (2% w/v FeNH₄(SO₄)₂ in 2 N HCl). The final assay mixture, containing both sample and assay solutions in a 2.5 mL volume was thus comprised (v/v) of 50.1% acetone, 33.5% butanol, 3.9% 12 N concentrated HCl, 7% dH₂O, 2.9% MeOH, and 2.6% ferrous reagent in a total volume of 2.5 mL. Aliquots (200 µL) of the final assay mixture of were removed to be read as non-heated controls. Assay samples were heated to 70°C for 2.5 h, allowed to cool to room temperature, and the absorbance read at 550 nm using a. To determine CT concentration, absorbances from unheated aliquots were subtracted from heated samples. When assaying water extracts for CTs, the proportion of assay reagent components, as well as volumes of extract and iron reagent, were adjusted to give the same water and solvent concentrations in the final assay mixture as for solvent-extracted CTs.

In Vitro Experiments

Experimental Animals

Two sheep of a local breed (Menz sheep) were treated with a single dose of commercial anthelmintic Levamisole (Manufacturer and country of Producer) at a dose of 10 mg/mL body weight (REF) and kept indoors at the Bahirdar University College of Agriculture and Veterinary Medicine (BUCAVM) farm. The absences of gastrointestinal parasites in feces of treated sheep were verified microscopically. The animals were examined for

nematode fecal egg production on the third and sixth days post anthelmintic treatment and all were negative for parasite eggs on the sixth day post treatment. Each animal was inoculated orally with 1750 *H. contortus* L₃ on the seventh day. The infective larvae were obtained by culturing *H. contortus* eggs collected from the previously mentioned two mono-species infected donor sheep. Eggs were collected after 4 weeks of infection and the eggs per gram were determined daily. The concentrations of eggs were estimated by counting the number of eggs in aliquots of 50 µL by the McMaster slide technique, as described by Cole.¹⁶ Our guideline is adopted from the Guide for the Care and Use of Agricultural Animals in Research and Teaching (Ag Guide). American Dairy Sciences Association, American Society of Animal Sciences and Poultry Sciences Associations, third edition, January 2010.¹⁷

Collection of Eggs

Briefly, fecal pellets were collected from the rectum of donor sheep and placed in a small bucket. Warm water was slowly added to the feces and the pellets stirred until a relatively liquid suspension was obtained. The suspension was mashed through a sieve with 3 mm aperture. The suspension that passed through the sieve was collected and washed through 100-mesh (150 µm pore size) sieve. The suspension was then poured in to 15 mL test tube and centrifuged for 2 min at 2000 RPM and the supernatant decanted. The tube was agitated by vortex mixer to loosen the sediment. Saturated sodium chloride was then added to the test tube until the meniscus forms above the tube on which the cover slip was placed. After 5 min the cover slip was carefully taken off the tube and the eggs washed in glass centrifuge tubes, filled with water and centrifuged for 2 min at 2000 RPM. Most of the water was then decanted and the number of eggs diluted to the required concentration for use in egg hatch assay (EHA), and larval development test (LDT).

Egg Hatch Assay (EHA)

The EHA was conducted according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines.¹⁸ Condensed tannin-enriched extracts of the plant leaves were used as the active treatment. Albendazole (pure standard reference) was used as positive control while untreated eggs in water were used as negative control. The test was conducted in 5-mL test tubes. In the assay, approximately

150–250 eggs in 1 mL of water were placed in each test tube. Various serial concentrations of 1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015 and 0.0078 mg/mL each plant extract in total volume of 0.5 mL in distilled water was added. Albendazole originally dissolved in dimethyl sulfoxide (DMSO) and diluted at concentrations of 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.0156 and 0.0078 µg/mL distilled water at different concentrations was used. The test tubes were covered and kept in an incubator at 27°C for 48 h. A drop of Lugol's iodine solution was added to each well to stop further hatching, and all the unhatched eggs and L1 larvae in each well were counted under dissecting microscope at 40x magnification. The experiment was replicated four times for each concentration and control.

Larval Development Test

The procedure used was a modification of the technique described previously.¹⁹ The experiment was conducted in plastic cups of 20 mL. The collected eggs were incubated at 27°C for 24 hours. An aliquot of 1 mL, containing 95–125 first-stage larvae (L₁) of *H. contortus*, was mixed with 5 g of feces collected from a sheep free of nematode eggs, and various serial concentrations of each plant extract. Serial concentrations of 50, 25, 12, 5, 6.25, 3.125 and 1.562 mg/mL of each plant were made in distilled water to make a total volume of 7 mL together with water containing L₁ and volume of egg-free feces. Ivermectin 1% (10 mg/mL) dissolved in 5% DMSO at concentrations of 0.5, 0.25, 0.12, 0.625, 0.3, 12, 0.15, and 0.078 mg/mL was used as positive control while untreated eggs in 5% DMSO was used as negative control. There were three replicates for each extract concentration and control. The plates were further incubated for 5 days (for a total of 7 days), and further development was stopped by the addition of one drop of Lugol's iodine solution: all L₁ and L₃ larvae in each well were counted under dissecting microscope at 40x magnification.

Statistical Analysis

The results of the in vitro tests were expressed as mean efficacy percentage of egg hatching or larval development inhibition ± standard deviation. The concentrations of the extracts required to inhibit 50% (IC₅₀) and 90% (IC₉₀) of egg hatching as well as larval development; and the relative median potency estimates of the condensed tannin extracts on egg hatchability larval development inhibition as compared to the positive control were calculated by probit analysis. Comparison of the

Table 1 Condensed Tannin Contents of the Extracts of Plants

Plant Samples	Condensed Tannin Contents Sorghum Tannin Equivalent (mg/ g of Extract)	95% CI	
		LB	UB
<i>A. gumifera</i>	7.2	2.13	12.27
<i>S. guineensa</i>	17.2	9.8	24.6
<i>R. glutinosa</i>	18.8	11.14	26.46

mean egg hatchability and larval development inhibition was carried out by a one-way ANOVA. $P < 0.05$ was considered statistically significant at 95% confidence level for all analysis.

Results

Phytochemical analysis and total tannin quantification of the extracts from the three plant samples were performed and the result indicated that *R. glutinosa* showed the highest tannin content and *A. gumifera* the least (Table 1). Meanwhile, the results of mean inhibition percentage (\pm SD) of condensed tannin-enriched extracts (Tables 2 and 3) showed that all three condensed tannin extracts

demonstrated various degrees of dose-dependent inhibition on both egg hatchability and larval development, with *R. glutinosa* being the highest followed by *S. guineensa*, whereas *A. gumifera* showed the lowest inhibition.

The IC_{50} and IC_{90} values of condensed tannin-enriched extract on egg hatchability and larval development inhibition is shown in Tables 4 and 5. Accordingly, the highest IC_{50} and IC_{90} values for egg hatchability and larval development inhibition were recorded with *A. gumifera* followed by *S. guineensa* whereas the lowest value was recorded with *R. glutinosa*. Hence, the condensed tannin inhibiting egg hatching and larval development most potentially was *R. glutinosa* followed in descending order of activity by *S. guineensa* and *A. gumifera*. This result suggests that all three condensed tannin-enriched extracts exhibited variation in potency to inhibit the egg hatchability and larval development.

Probit analysis was used to compare egg hatchability and larval development inhibition of the condensed tannin extracts by comparing their relative potency with that of the standard counterparts (positive controls); thus, *R. glutinosa* was 3.7 and 5.9 times more potent in inhibiting egg hatchability than *S. guineensa* and *A. gumifera*,

Table 2 Mean Inhibition Percentage (\pm SD) of Different Concentrations of Condensed Tannin Extracts on Egg Hatchability of Sheep *H. contortus*

Concentration (mg mL ⁻¹)	Condensed Tannins and Positive Control			
	<i>A. gumifera</i>	<i>S. guineensa</i>	<i>R. glutinosa</i>	Albendazole
0.0156	1.64 \pm 1.75	2.07 \pm 2.01	3.26 \pm 2.50	3.82 \pm 2.54
0.0312	2.93 \pm 2.46	3.14 \pm 2.14	3.29 \pm 2.52	7.07 \pm 3.66
0.0625	3.35 \pm 2.50	6.15 \pm 3.43	7.23 \pm 3.61	15.99 \pm 4.83
0.125	10.9 \pm 4.01	15.68 \pm 5.01	22.87 \pm 5.76	81.46 \pm 4.99
0.25	25.07 \pm 5.82	30.33 \pm 6.20	59.47 \pm 6.55	100 \pm 0.00
0.5	52.07 \pm 6.65	57.33 \pm 6.41	87.34 \pm 4.31	100 \pm 0.00
1	78.41 \pm 5.35	88.62 \pm 4.35	99.08 \pm 1.39	100 \pm 0.00

Table 3 Mean Inhibition Percentage (\pm SD) of Different Concentrations of Condensed Tannin Extracts on Larval Development of Sheep *H. contortus*

Concentration (mg mL ⁻¹)	Condensed Tannins and Positive Control			
	<i>A. gumifera</i>	<i>S. guineensa</i>	<i>R. glutinosa</i>	Ivermectin
1.562	29.72 \pm 8.66	36.63 \pm 9.09	43.52 \pm 9.35	46.89 \pm 9.24
3.125	38.51 \pm 9.22	48.10 \pm 8.87	55.59 \pm 9.29	58.41 \pm 9.43
6.25	50.00 \pm 9.75	61.95 \pm 8.95	66.77 \pm 8.8	80.52 \pm 8.25
12.5	61.67 \pm 9.53	70.81 \pm 8.61	78.70 \pm 7.58	82.12 \pm 7.16
25	73.27 \pm 8.23	78.89 \pm 7.52	86.25 \pm 6.56	89.81 \pm 5.81
30	84.16 \pm 5.67	89.41 \pm 5.67	91.07 \pm 5.28	93.91 \pm 4.37

Table 4 The IC₅₀ and IC₉₀ in mg mL⁻¹ of the Condensed Tannin Extracts on Egg Hatchability Inhibition of Sheep *H. contortus*

Condensed Tannins	IC ₅₀ (mg mL ⁻¹)	95% CI		IC ₉₀ (mg mL ⁻¹)	95% CI	
		LB	UB		LB	UB
<i>A. gumifera</i>	0.5	0.36	0.68	1.65	1.15	2.76
<i>S. guineensa</i>	0.41	0.3	0.54	1.19	0.87	1.87
<i>R. glutinosa</i>	0.21	0.17	0.26	0.49	0.39	0.68
Albendazole	0.08	0.06	0.11	0.23	0.17	0.37

Table 5 The IC₅₀ and IC₉₀ in mg mL⁻¹ of the Condensed Tannin Extracts on Larval Development Inhibition of Sheep *H. contortus*

Condensed Tannins	IC ₅₀ (mg mL ⁻¹)	95% CI		IC ₉₀ (mg mL ⁻¹)	95% CI	
		LB	UB		LB	UB
<i>A. gumifera</i>	5.89	4.08	8.11	106.41	69.59	184.47
<i>S. guineensa</i>	3.45	2.36	4.73	62.22	42.58	100.88
<i>R. glutinosa</i>	2.27	1.51	3.16	39.34	27.76	60.9
Ivermectin	0.66	0.38	1.03	11.85	8.68	16.73

respectively. Similarly, *R. glutinosa* was 2.5 and 7.3 times more potent in inhibiting larval development than *S. guineensa* and *A. gumifera*, respectively (Tables 6–8).

However, Tukey's HSD post-hoc test revealed that the observed difference in the mean egg hatchability and larval development inhibition between *A. gumifera* (mean = 62.93, SD 72.50) and albendazole (mean = 118.56, SD 98.71) was not statistically significant ($P = 0.24$). Similar results with their corresponding descriptive and ANOVA values were observed in Tables 9–11 pertaining to *S. guineensa* and *R. glutinosa*.

Discussions

The present study was aimed at investigating the direct effects of condensed tannins on egg hatchability and larval development inhibition of *H. contortus* of sheep.

Table 6 Relative Median Potency Estimates of the Condensed Tannin Extracts on Egg Hatchability Inhibition of Sheep *H. contortus* as Compared to the Positive Control

Condensed Tannins and Control	Estimates	95% CI	
		LB	UB
<i>A. gumifera</i>	0.16	0.04	0.39
Albendazole	6.12	0.258	25.04
<i>S. guineensa</i>	0.2	0.05	0.45
Albendazole	4.94	2.23	18.6
<i>R. glutinosa</i>	0.39	0.19	0.63
Albendazole	2.54	1.58	5.42

Table 7 Relative Median Potency Estimates of the Condensed Tannin Extracts on Larval Development Inhibition of Sheep *H. contortus* as Compared to the Positive Control

Condensed Tannins and Control	Estimates	95% CI	
		LB	UB
<i>A. gumifera</i>	0.11	0.06	0.19
Ivermectin	8.95	5.22	8.09
<i>S. guineensa</i>	0.19	0.11	0.3
Ivermectin	5.23	3.31	9.3
<i>R. glutinosa</i>	0.3	0.18	0.45
Ivermectin	3.35	2.21	5.52

In view of that, three indigenous medicinal plants were selected for this study based on their relatively high content of condensed tannins. The effect of condensed

Table 8 Relative Median Potency Estimates of the Condensed Tannin Extract on Larval Development Inhibition of Sheep *H. contortus* as Compared to the Positive Control

Condensed Tannins and Control	Estimates	95% CI	
		LB	UB
<i>A. gumifera</i>	0.11	0.06	0.19
Ivermectin	8.95	5.22	8.09
<i>S. guineensa</i>	0.19	0.11	0.3
Ivermectin	5.23	3.31	9.3
<i>R. glutinosa</i>	0.3	0.18	0.45
Ivermectin	3.35	2.21	5.52

Table 9 One-Way ANOVA for Egg Hatchability and Larval Development Inhibition Test of *A. gumifera* Against Sheep *H. contortus*

Egg Hatching Inhibition Assay						
	Treatment	N*	Mean Egg	SD	95% CI	
			Hatchability		Lower Bound	Upper Bound
			Inhibition			
Descriptive	(A) <i>gumifera</i> Albendazole	8 8 Sum of Seq.	62.93 118.56 <i>df</i>	72.5 98.71 Mean Eq.	7.2 42.68 <i>F</i>	118.66 194.43 <i>P</i> -value
ANOVA	Between group Within group	63,329.24 119,996.17	2 24	31,664.62 4999.84	6.33	0.006
Larval Development Inhibition Test						
	Treatment	N*	Mean Larval	SD	95% CI	
			Developmental		Lower Bound	Upper Bound
			Inhibition			
Descriptive	<i>A. gumifera</i> Ivermectin	7 7 Sum of Seq.	51.9 76.86 <i>df</i>	29.03 36.53 Mean Seq.	25.06 43.07 <i>F</i>	78.75 110.64 <i>P</i> -value
ANOVA	Between group within group	21,522.07 13,061.68	2 18	10,761.04 725.65	14.83	0.0001

Note: *The total number of experimental animals.

Table 10 One-Way ANOVA for Egg Hatchability and Larval Development Inhibition Test of *S. guineensa* Against Sheep *H. contortus*

Egg Hatching Inhibition Assay						
	Treatment	N*	Mean Egg	SD	95% CI	
			Hatchability		Lower Bound	Upper Bound
			Inhibition			
Descriptive	<i>S. guineensa</i> Albendazole	8 8 Sum of Seq.	66.41 118.55 <i>df</i>	74.51 98.71 Mean Eq.	9.13 42.68 <i>F</i>	123.68 194.43 <i>P</i> -value
ANOVA	Between group Within group	63,554.38 122,360.62	2 24	31,777.19 5098.36	6.23	0.007
Larval Development Inhibition Test						
	Treatment	N*	Mean Larval	SD	95% CI	
			Developmental		Lower Bound	Upper Bound
			Inhibition			
Descriptive	<i>S. guineensa</i> Ivermectin	7 7 Sum of Seq.	63.05 76.86 <i>df</i>	32.26 36.53 Mean Seq.	33.21 43.07 <i>F</i>	92.89 110.64 <i>P</i> -value
ANOVA	Between group Within group	23,503.03 14,252.95	2 18	11,751.51 791.83	14.84	0.0001

Table 11 One-Way ANOVA for Egg Hatchability and Larval Development Inhibition Test of *R. glutinosa* Against Sheep *H. contortus*

Egg Hatching Inhibition Assay						
	Treatment	N*	Mean Egg	SD	95% CI	
			Hatchability		Lower Bound	Upper Bound
			Inhibition			
Descriptive	<i>R. glutinosa</i>	8	85.74	86.16	19.52	151.97
	Albendazole	8	118.55	98.71	42.68	194.43
	Distilled water	8	0.001	0.001	0.001	0.001
	Sum of Seq.		<i>df</i>	Mean Seq.	<i>F</i>	<i>P</i> -value
ANOVA	Between Group within group	67,451.12 137,325.06	2 24	33,725.56 5721.88	5.89	0.008
Larval Development Inhibition Test						
	Treatment	N*	Mean Larval	SD	95% CI	
			Developmental		Lower Bound	Upper Bound
			Inhibition			
Descriptive	<i>R. glutinosa</i>	7	66.43	34.9	34.16	98.7
	Ivermectin	7	76.86	36.53	43.07	110.64
	Distilled water	7	0.001	0.001	0.001	0.001
	Sum of Seq.		<i>df</i>	Mean Seq.	<i>F</i>	<i>P</i> -value
ANOVA	Between group within group	24,333.238 15,313.016	2 18	12,166.62 850.72	14.3	0.0001

Note: *The total number of experimental animals.

tannin extracts which was demonstrated in our study is in accord with a series of in vitro studies that supported the anthelmintic property of condensed tannins.^{8,15,20–22}

Demonstration of various degrees of dose-dependent inhibition on both egg hatchability and larval development by all condensed tannin extracts is in agreement with previous studies by different authors.^{20,23–25} There are two hypotheses proposed to elucidate the anthelmintic effects of condensed tannins. Primarily, the direct hypothesis: the ability of these compounds to interact with proteins of the cuticle, oral cavity, esophagus, cloaca and vulva of nematodes, changing their chemical and physical properties. Secondly, the indirect hypothesis: the capacity of condensed tannins to bind dietary proteins and protect them from rumen degradation increasing protein flow to and amino acid absorption by the small intestine, improving host immune response against worms.^{26,27}

The effective doses (IC₅₀ and IC₉₀) are defined as the concentration of drug or extract producing 50% and 90%, respectively, inhibition on egg hatching or larval development.²⁸ Consequently, the three condensed tannin

extracts in this study revealed a range of efficacies to inhibit egg hatching and larval development. The observed differences in potencies among the extracts might be associated with the corresponding variation in their tannin contents. Related work with the in vitro inhibitory effect of condensed tannins on egg hatchability and larval development of *Haemonchus contortus* was reported previously.²¹

It has been stated that controls of *Haemonchus contortus* could not be resolved by the use of conventional anthelmintic drugs²¹ as there is worldwide problem regarding the development of anthelmintic-resistant worm populations. The three species of plants were chosen for our trial based on their relatively high tannin content and their wide availability in the study area. The promising result of the present study is that all three plants exhibited various dose-dependent in vitro efficacies on egg hatchability and larval development of *H. contortus* in sheep. This could provide baseline information on the possibility of considering condensed tannins as an alternative in the packages towards the control of haemonchosis in sheep.

The in vitro ovicidal and larvicidal action of condensed tannin plant extracts on eggs and larvae of *H. contortus* of sheep may not imply that the extracts would have similar action on adult worm parasites. The relevance of in vitro studies to in vivo efficacy, in regard to anthelmintic activity, is greatly influenced by differences in the physiology and bioavailability of plant preparations within the host.²⁹

Conclusion

All three condensed tannin extracts demonstrated various degrees, yet very close dose-dependent inhibition of both egg hatchability and larval development. According to IC₅₀ and IC₉₀ values, the condensed tannin inhibiting egg hatching and larval development most potently was *R. glutinosa* followed in descending order of activity by *S. guineensis* and *A. gumifera*. The result of the present study suggests that condensed tannins might be used as an option for the control of *Haemonchus contortus* of sheep.

Data Sharing Statement

Data will be made available upon request of the primary author.

Ethics Statement

For this study, ethical clearance and statement were given for the research team members with the help of the University of Gondar. The study has been reviewed by the Institutional Ethical Review Board of University of Gondar for its ethical soundness, and it is found to be ethically acceptable. Thus, the Research and Community Service Vice President Office has awarded on R.NO.O/V/RCS/05/8/3/2018. Our guideline is adopted from Guide for the Care and Use of Agricultural Animals in Research and Teaching (Ag Guide).

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Author Contributions

MB, AK, HD, MY and TG are substantial contributors to the conception and design, acquisition of data, or analysis and interpretation of data; drafting the article or revising it critically for important intellectual content; final approval of the version to be published and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Disclosure

The authors declare that they have no competing interests for this work.

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