Global Veterinaria 11 (6): 759-766, 2013
ISSN 1992-6197
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DOI: 10.5829/idosi.gv.2013.11.6.76201

Comparison of the Efficacy of Different Modes of Extraction of 5 Tannin Rich Plants on *Haemonchus contortus*: Searching for Indicators Based on A Range of *In vitro* assays

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Abstract: The study was undertaken to evaluate effects of condensed tannin concentration (CT) on *in vitro* inhibitory effects of CT on egg hatchability, larvae development and adult mortality of *Haemonchus contortus* (*H. contortus)*. The CT sources included were *Albizia gummifera, Carissa edulis, Ficus ovata, Maytenus obscura* and *Rhus glutinosa*. Aqueous acetone and ethanol (each having 50 and 70% v/v dose), were incorporated for the extraction of CT. Distill water and Albendazole, commercial anthelmintics drug, were used as negative and positive control, respectively. The CT extracts induced anthelmintic effects on the three stages of *H. contortus* and these effects were significantly different when they were compared to the positive and negative control group (P<0.001). The fastest and slowest mortality rates were recorded for ethanol (2:08) and acetone (4:25 to 4:27 h) dosages in the fresh leaves of *A. gummifera*, respectively (P<0.001). Although all CT rich extracts showed anti- *H. contortus* activity, the inhibiting efficiency was depending on the CT concentration. Thus, forages containing CT could offer a nutritionally-based ecologically sustainable system for controlling the effects of parasites such as *H. contortus*.

Key words: Anthelmintics · *H. contortus* · Parasite · Tannin · Tannin rich plants

INTRODUCTION

The major animal diseases in the humid tropics are mostly due to gastro-intestinal (GI) parasitism (e.g., *Haemonchus, Bunostomum*) [1-3]. 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 [4, 5]. The high cost of pharmaceutically derived anthelmintic drugs, residual concern in food animals and environmental pollution have stirred up interest in medicinal plants as an alternative source of anthelmintic drugs [6-8]. Moreover, the current efficacy of these drugs has been reduced, because of widespread application of poor quality synthetic anthelmintics and consequently the development of resistant parasite strains [9-11]. Tannin extractives from foliages of tropical fodder trees can be the best strategy to use as alternative strategy for controlling helminthes. Hence, the use of indigenous plant preparations as livestock deformers is gaining ground as one of the options and sustainable methods readily adapted to rural farming communities [12, 13]. This is the first research work that evidences the interaction effects of tannin sources and doses of extraction solvents in relation to *in vitro* anti-gastro-intestinal activities. The study was planned to quantify and compare the *in vitro* anti- *H. contortus* activity of condensed tannins extracts of five fodder trees.

MATERIALS AND METHODS

Collection of Plant Materials and Tannin Extraction Protocol: The tannin rich plant species [14] such as *Albizia gummifera (*CT = 7.2%*), Carissa edulis* (CT = 16.4%*), Ficus ovata* (CT = 19.1%*), Maytenus obscura* (CT = 22.8%) and *Rhus glutinosa* (CT = 18.8%) were

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collected from their natural habitat, Omo Nada district of Jimma zone, Ethiopia. The collected leaves were put in plastic bags and directly placed on ice in insulated container and transported under dark conditions to Jimma University animal nutrition laboratory within 30 minutes. Immediately after arrival half of the fresh leaves were extracted to 1 mm sieve (FLE) and the other halves were dried at approximately 55°C to constant weight using air forced oven and ground to 1mm (FLD). Leaf samples dried, ground and preserved for 1.5 years (FLP) at room temperature (avg., 20 °C) were also used for this particular study. All FLE, FLD and LDP leaf materials (200 mg) were used for tannin extraction. The complete tannin extraction process for all the aqueous acetone (50 and 70%) and ethanol (50% and 70%) doses was followed IAEA/FAO [15]. The extract was stored at 4°C until used. Because in several trials, the effect of using acetone or ethanol alone as control had no significant effect on anthelmintics activity [14], thus, acetone and ethanol were not used without plant extract.

Collection of Adult Parasite, Eggs and Third Stage Larvae of *H, contortus:* *H. contortus* adult worms were obtained from carcasses following meat inspection looking for adult worms at specific predilection site (abomasa) from abattoir house [16]. The egg recovery was performed according to the method described by Jabbar *et al*. [17] where female adult worms were crushed using pestle and mortar. After liberation, the eggs were cultured in a 250 ml jar filled with autoclaved sheep faeces for 16 days at 26-28°C [17].

Experimental infection of sheep with *H. contortus*: About 2500 third stage larvae were inoculated to six dewormed Menz sheep breed of Ethiopia. The infected sheep was kept in a partitioned animal house of the Faculty of Veterinary Medicine, AU. The sheep was served as donor of *H. contortus* eggs for the *in-vitro* tests.

Collecting and Counting Eggs from Donor Sheep: Faeces that were collected from the donor sheep were processed and the filtrate was centrifuged in test tubes for 1 minute at 2000 rpm and supernatant was discarded. Tubes were then agitated on a vortex mixer to loosen the sediment and saturated NaCl solution was added until a meniscus formed above the tube. A cover slip was placed and plucked off carefully after 5 minutes from tubes and eggs were washed off into a conical glass centrifuge tube. The tubes were filled with water and centrifuged for 1 minute at 2000 rpm. The supernatant was decanted and eggs were re-suspended in saline solution. The concentration of recovered egg samples were determined using a modified McMaster technique where the sample solution was placed into one half of a McMaster slide. The number of eggs counted in both sides of the chamber was multiplied by 50 to estimate the total number of eggs in the sample. The result was reported as egg per gram (EPG) as according to Coles *et al*. [18].

Egg Hatchability Inhibition: The egg hatchability inhibition test was conducted according to the procedure described by Coles *et al.* [18]. Extracts of CT from the five plant species was used as the test treatments. Albendazole dissolved in Dimethyl-Sulfoxide (DMSO) and diluted in distilled water was used as a positive control while an untreated egg in distilled water was used as negative control. The test was conducted in 10 ml test tubes. In the assay, approximately 170-270 eggs in 1.5 ml of water were place in each test tube. About 50 mg/ml of each plant extract was added in to test tubes. The test tubes were covered with aluminum foil making 15 to 20 holes for air circulation and kept in an incubator at 27°C for 48 hrs. The experiment was repeated three times. Hatched larvae and unhatched eggs were then counted under dissecting microscope at 40 × magnification.

The anti- *H. contortus* efficacy of CT was assessed by field controlled faecal egg count reduction test calculated according to Coles *et al*. [18] and Taylor *et al.* [19]. The percent faecal egg count reduction was calculated using the following formula:

$$\% FECR = (a-b) \times 100/a$$

where, *a,* EPG pre-treatment and *b*, EPG post treatment

Larval Development Inhibition Assay: The larval development inhibition test was conducted with a modification of the technique described by Costa *et al*. [20]. The CT extracts from the five plant species were used as test treatments. Albendazole 10 mg/ml dissolved in DMSO and diluted in distilled water used as positive control while untreated eggs in distilled water were used as negative control. After incubating the eggs at 27°C for 24 hours, an aliquot of 1.5ml, containing 100-150 first stage larvae (L_1) of *H. contortus* was mixed with 10 gm of faeces that collected from a de-wormed sheep free of gastrointestinal nematodes. A 50 mg/ml concentration of each CT extract was added on a sheep faeces which containing L_1. The test materials were incubated for 6 days at room temperature. At the end of 6th day the wall of

each cup containing the sample was thoroughly rinsed with 10 ml of water to collect the larvae. Then one drop of Lugol's iodine solution was added and all (L_3) stage larvae were counted under stereomicroscope.

Adult Motility Test: Adult motility test was performed according to Petersen *et al*. [21]. Accordingly 50 mg/ml of each CTs extracts was diluted with distilled water. Albendazole dissolved in DMSO and diluted in distilled water was used as a positive control while distilled water was used as negative control. For each treatment 10 adult parasites were used. The same concentration form each plant extracts (50 mg/ml) was placed in petri-dish, the parasites were immersed and the times of mortality were recorded.

Statistical Analysis: A variance analysis model with four fixed factors was used following the general linear model (GLM) procedure of statistical analysis system [22]: plant species (P; 5 levels), storage conditions (S; 3 levels), extraction solvents (M; 2 levels) and dosage of extraction solvents (D; 2 levels) in 5 × 3 × 2 × 2 factorial arrangement.. The overall interactions were used to interpret the analyses. Differences between means were tested using least significance difference with significances declared at $P < 0.05$.

RESULTS

Egg Hatchability Inhibition: The mean inhibition percentage obtained for each extraction solvent was significantly different with differences in storage condition and doses of extraction solvents ($P<0.001$) (Table 1). The mean inhibition percentage of all plant extracts for acetone 50% varied from the lowest inhibition effect at dried *R. glutinosa* (13.1%) to the highest inhibition effect of dried-preserved *F. ovata* (76.6%) were significant different along the three different storage times ($P<0.001$). Comparing the inhibition effect of acetone 50% for fresh leaf extractives of *M. obscura* presented the highest inhibition activity (60.9%) than the leaf extracts of *C. edulis* (52.3%), *R. glutinosa* (44.6%), *F. ovata* (40.9%) and *A. gummifera* (37.4%)($P<0.001$). In contrast, acetone 70% extract for *C. edulis* (55.3%) and *M. obscura* (9.1%) leaves had the highest and lowest values of egg inhibition effect across the storage conditions and plant species, respectively ($P<0.001$). Whereas the highest (75.4%) and lowest (21.3%) % egg inhibitions were recorded for *R. glutinosa* and *A. gummifera* leaves at fresh state extracted by 50% ethanol, respectively ($P<0.001$). Further, the highest % egg inhibitions by ethanol 70% extracts was determined for *C. edulis* and *F. ovata* leaves at fresh condition whereas the lowest % egg inhibition effect was recorded for fresh dried leaves of *C. edulis* ($P<0.001$).

Comparison between extraction solvents for storage conditions of the plant species on % egg inhibition are presented in Table 2. The highest % egg inhibition of *A. gummifera* was observed at 70% acetone extraction procedure across the storage conditions of the same plant as compared to % egg inhibition capacity of the rest of organic solvents ($P<0.001$). On the other hand, across the storage conditions in *C. edulis* the highest % egg inhibition was recorded for 50% acetone as compared to the rest of the extraction solvents ($P<0.001$).

Larval Development Inhibition: The experiment was done with exposure of *H. contortus* L_1 to the extracts of the experimental plant species in a 50 mg/ml dose for larva development test (Table 3). Condensed tannins inhibited larval development by 100%. In addition, in *C. edulis* and *R. glutinosa* at all storage conditions the effectiveness of CT on larval development was also by 100%. It was also observed in most of storage conditions in the plant species that the larval development inhibition of the CT was ranged from 2 to 100% in 70% acetone extraction. In comparison to acetone 50 and 70%, CT extracted with Ethanol 50% inhibited larval development to the maximum 100% only in six out of total fifteen storage conditions.

Condensed tannins extracted with 70% ethanol from *A. gummifera* inhibited larval development by 100% in all storage conditions as compared to storage conditions of other plant species ($P<0.001$).

Least square means for the effects of storage condition of the plant species compared separately for extraction solvents on % larval development inhibition is presented in Table 4. Condensed tannin from fresh leaves extracted with acetone 50% and ethanol 70% inhibited larval development by 100% as compared to acetone 70% and ethanol 50% in *A. gummifera* ($P<0.001$). However, the effects of extraction solvents didn't vary in inhibiting larval development activity in fresh leaves of *F. ovata.* The larval development inhibiting ability of CT extracted by various organic solvents varied with differences in plant species and in most of the plants the 100% inhibition was recorded for fresh while for the others the highest value was recorded at dried-preserved storage conditions.

Table 1: Least square means for the effects of storage condition of plant species and extraction solvents on % egg inhibition compared for each extraction solvent

	Albizia gummifera			*Carissa edulis*			*Ficus ovata*			*Maytenus obscura*			*Rhus glutinosa*				
ES	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP	SE	*P*-value
A 50%	37.4^{j}	42.6^{h}	36.0^{k}	52.3^{f}	57.4^{c}	59.4^{d}	40.9^{i}	61.4^{b}	76.6^{a}	60.9^{c}	15.2^{n}	32.9^{j}	44.6^{g}	13.1^{o}	30^{m}	0.02	<0.001
A 70%	49.1^{b}	48.9^{b}	47.7^{c}	14^{m}	55.3^{a}	34.3^{i}	41.7^{c}	41.1^{f}	38^{g}	20.6^{l}	23.1^{k}	9.1^{n}	44.9^{d}	32.9^{j}	35.7^{h}	0.22	<0.001
E 50%	44.3^{c}	21.3^{o}	34.5^{f}	28^{j}	34.9^{e}	28.6^{i}	38.6^{d}	27.1^{l}	23.4^{n}	33.2^{h}	33.7^{g}	27.4^{k}	75.4^{a}	25.7^{m}	61.1^{b}	0.02	<0.001
E 70%	42.3^{d}	27.7^{g}	12.9^{k}	50.9^{a}	6.6^{l}	18.6^{j}	57.4^{a}	23.1^{i}	20^{j}	26^{h}	46.9^{b}	38.3^{e}	44.6^{c}	22.3^{i}	32.3^{f}	0.58	<0.001

Table 2: Least square means for the effects of storage condition of plant species and extraction solvents on % egg inhibition compared between extraction solvents

	Albizia gummnifera			*Carissa edulis*			*Ficus ovata*			*Maytenus obscura*			*Rhus glutinosa*		
ES	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP
A 50%	37.4^{d}	42.6^{c}	36.0^{b}	52.3a	57.4^{a}	59.4^{a}	40.9^{c}	61.4^{a}	76.6^{a}	60.9^{a}	15.2^{d}	32.9^{b}	44.6^{c}	13.1^{d}	30^{d}
A 70%	49.1^{a}	48.9^{a}	47.7^{a}	14^{d}	55.3^{b}	34.3^{b}	41.7^{b}	41.1^{b}	38^{b}	20.6^{d}	23.1^{c}	9.1^{d}	44.9^{b}	32.9^{a}	35.7^{b}
E 50%	44.3^{b}	21.3^{d}	34.5^{c}	28^{c}	34.9^{c}	28.6^{c}	38.6^{d}	27.1^{c}	23.4^{c}	33.2^{b}	33.7^{b}	27.4^{c}	75.4^{a}	25.7^{b}	61.1^{a}
E 70%	42.3^{c}	27.7^{c}	12.9^{d}	50.9^{a}	6.6^{d}	18.6^{d}	57.4^{a}	23.1^{d}	20^{d}	26^{c}	46.9^{a}	38.3^{a}	44.6^{c}	22.3^{c}	32.3^{c}
SE	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.30	0.02	0.02	0.02	0.02	0.02	0.02
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PC	100														
NG	5.5														

*F, fresh leaves; FD, dried; DP, preserved; PS, plant species; ES, extraction solvents; ST, storage time; A, acetone; E, ethanol; PC, positive control; NG, negative control; SE, standard error of mean;***P<0.001*

Table 3: Least square means for the effects of storage condition of the plant species compared separately for each extraction solvent on % larva development inhibition test

	Albizia Gummifera			*Carissa edulis*			*Ficus ovata*			*Maytenus obscura*			*Rhus glutinosa*				
ES	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP	SE	P
A 50%	100^{a}	100^{a}	3.3^{d}	100^{a}	100^{a}	100^{a}	100^{a}	83.3^{b}	100^{a}	100a	17.3^{c}	100^{a}	100^{a}	100^{a}	100^{a}	0.50	<0.001
A 70%	9.3^{e}	100^{a}	100^{a}	100^{a}	100^{a}	4.7^{f}	100^{a}	2^{g}	100^{a}	100^{a}	87.3^{c}	90.9^{b}	64.7^{d}	100^{a}	2.7^{g}	0.37	<0.001
E 50%	62^{c}	16.7^{g}	100^{a}	12^{i}	14.7^{h}	80^{c}	100^{a}	87^{b}	100^{a}	6^{j}	48.7^{f}	100^{a}	74^{d}	100^{a}	100^{a}	0.49	<0.001
E 70%	100^{a}	100^{a}	100^{a}	100^{a}	12^{e}	100^{a}	100^{a}	100^{a}	3.3^{g}	6.7^{f}	100^{a}	41.3^{e}	43.3^{b}	30.7^{c}	100^{a}	0.38	<0.001

*F, fresh leaves; FD, dried; DP, preserved; PS, plant species; ES, extraction solvents; ST, storage time; A, acetone; E, ethanol; SE, standard error of mean;***P<0.001*

Table 4: Least square means for the effects of storage condition of the plant species compared separately for extraction solvents on % larva development inhibition

	Albizia gummifera			*Carissa Edulis*			*Ficus ovata*			*Maytenus obscura*			*Rhus glutinosa*		
ES	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP
A 50%	100^{a}	100^{a}	3.3^{b}	100^{a}	100^{a}	100^{a}	100	83,3^{c}	100^{a}	100^{a}	17.3^{c}	100^{a}	100^{a}	100^{a}	100^{a}
A 70%	9.3^{c}	100^{a}	100^{a}	100^{a}	100^{a}	4.7^{c}	100	2^{d}	100^{a}	100^{a}	87.3^{b}	90.9^{b}	64.7^{c}	100^{a}	2.7^{b}
E 50%	62^{b}	16.7^{b}	100^{a}	12^{b}	14.7^{b}	80^{b}	100	87^{b}	100^{a}	6^{b}	48.7^{c}	100^{a}	74^{b}	100^{a}	100^{a}
E 70%	100^{a}	100^{a}	100^{a}	100^{a}	12^{c}	100^{a}	100	100^{a}	3.3^{b}	6.7^{b}	100^{a}	41.3^{c}	43.3^{d}	30.7^{b}	100^{a}
SE	0.43	0.43	0.43	0.43	0.35	0.35	0.43	0.37	0.43	0.5	0.25	0.35	0.25	0.43	0.5
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	NS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PC	100														
NC	4.6														

*F, fresh leaves; FD, dried; DP, preserved; PS, plant species; ES, extraction solvents; ST, storage time; A, acetone; E, ethanol; PC, positive control; NG, negative control; SE, standard error of mean;***P<0.001*

Table 5: Least square means for the effects of plant species and storage time for each extraction solvents on adult motility

	Albizia Gummnifera			*Carissa Edulis*			*Ficus Ovata*			*Maytenus obscura*			*Rhus glutinosa*				
ES	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP	SE	P
A 50%	$4:25^{a}$	$2:38^{k}$	$3:50^{c}$	$3:40^{e}$	4.20^{b}	$2:40^{j}$	$2:52^{h}$	$1:45^{n}$	$2:08^{m}$	$2:45^{i}$	$2:.30^{l}$	$3:40^{e}$	$3:05^{g}$	$3:46^{d}$	$3:15^{f}$	0.005	<0.001
A 70%	$4:27^{a}$	$2:30^{j}$	$3:45^{d}$	$4:08^{c}$	$4:20^{b}$	$2:46^{h}$	$2:20^{l}$	$2:35^{i}$	$2:25^{k}$	$2:35^{i}$	$1:45^{m}$	$3:35^{e}$	$2:50^{g}$	$3:45^{d}$	$3:25^{f}$	0.005	<0.001
E 50%	$2:08^{i}$	$2:25^{g}$	$3:47^{b}$	$3;50^{a}$	$2:35^{f}$	$2:45^{e}$	$2:10^{b}$	$1:46^{j}$	$2:25^{g}$	$1:30^{k}$	$1:45^{j}$	$3:30^{c}$	$2:50^{d}$	$2:45^{e}$	$2:50^{d}$	0.005	<0.001
E 70%	$2:08^{h}$	$2:10^{g}$	$2:35^{d}$	$3:10^{g}$	$2:36^{d}$	$2:36^{d}$	$2:22^{e}$	$1:50^{i}$	$2:08^{h}$	$2:20^{f}$	$1:50^{i}$	$3:05^{b}$	$2:45^{c}$	$2:35^{d}$	$3:52^{a}$	0.004	<0.001

*F, fresh leaves; FD, dried; DP, preserved; PS, plant species; ES, extraction solvents; ST, storage time; A, acetone; E, ethanol; PC, SE, standard error of mean;***P<0.001*

Table 6: Least square means for the effects of plant species, extraction solvents and storage time on adult motility

	Albizia gummifera			*Carissa Edulis*			*Ficus ovata*			*Maytenus obscura*			*Rhus glutinosa*		
ES	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP
A 50%	4:25^{b}	2:38^{a}	3:50^{a}	3:40^{a}	4:20^{a}	2:40^{c}	2:52^{a}	1:45^{d}	2:08^{b}	2;45^{a}	2:30^{a}	3:40^{a}	3:05^{a}	3:46^{a}	3:15^{c}
A 70%	4:27^{a}	2:30^{b}	3:45^{c}	4:08^{a}	4;20^{a}	2:46^{a}	2:20^{c}	2:35^{a}	2:25^{a}	2:35^{b}	1:45^{c}	3:35^{b}	2:50^{b}	3:45^{b}	3:25^{b}
E 50%	2:08^{c}	2:25^{c}	3:47^{b}	3:50^{b}	2:35^{c}	2:45^{b}	2:10^{d}	1:46^{c}	2:25^{a}	1:30^{d}	1:45^{c}	3:30^{c}	2:50^{b}	2:45^{c}	2:50^{d}
E 70%	2:08^{c}	2:10^{d}	2:35^{d}	3:10^{d}	2:36^{b}	2:36d	2:22^{b}	1:50^{b}	2:08^{b}	2:20^{c}	1:50^{b}	3:05^{d}	2:45^{c}	2:35^{d}	3:52^{a}
SE	0.0025	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PC	2:33														
NG	5:40														

F, fresh leaves; FD, dried; DP, preserved; PS, plant species; ES, extraction solvents; ST, storage time; A, acetone; E, ethanol; PC, positive control; NG, negative control; SE, standard error of mean; ***P<0.001*

Adult Motility Test: The experiment was done with the exposure of 10 adult *H. contortus* adult parasite, to the experimental plant species using 50 mg/ml dose for adult motility test (Table 5). This table presents adult parasite mortalities in hours; the larger number of hours indicated the slower mortality rate of the parasite. In other words, the lower time indicates the fastest motility rate. The effects of plant species and storage condition showed different adult motility rate across the extraction solvents (P<0.001). The fastest adult motility rate was observed in *F. ovata* at fresh dried storage condition (1:45 h) whereas the slowest adult motility rate was recorded in *A. gummifera* at fresh storage condition with acetone 50% extraction (P<0.001). On the other hand, the fastest rate of adult mortality was observed for *M. obscura* (1:45 h) at fresh dried storage condition whereas the slowest mortality rate (4:27 h) was recorded for *A. gummifera* leaves extracted with acetone 70% at fresh condition. The fastest adult motility rate (for the CT concentration obtained through extracting with ethanol 50%) was recorded from the fresh leaves of *M. obscura* (1:30 h) and the slowest mortality (3:50 h) was observed in *C. edulis* at fresh condition (P<0.001). The CT values extracted with Ethanol 70% had the fastest adult motility rate both in *F. ovata* (1:50 h) and *M. obscura* (1:50 h) at fresh dried storage condition whereas the slowest mortality was recorded in *R. glutinosa* (3:52 h).

Statistically significant difference was observed in adult mortality time across the storage conditions and extraction solvents for each tanniferous species (P<0.001) (Table 6). The fastest and the slowest mortality rate was recorded for Ethanol (2:08) and acetone (4:25 to 4:27 h) levels in the fresh leaves of *A. gummifera*, respectively (P<0.001). In general in *C. edulis*, the adult motility rate ranged from 2:35 until 4:20; for *F. ovata*, the highest and the lowest adult mortality rate was recorded at fresh (2:52) and fresh dried (1:45 h)(P<0.001).

DISCUSSION

Generally from Table 1, it's observed that different values of % egg hatching inhibition for the plant species at various storage conditions and dosages of the extraction solvents. This variation might be correlated with variation in genetic factors and biochemical activity of the plant. The assumption is that condensed tannins in tanniferous plant extracts can complex with the sheath proteins of nematodes, which have high proline content, prevent exsheathment and intercalate with DNA synthesis of parasites [23]. However, the exact mechanisms of action of these metabolites remain obscure and could differ depending on the parasite, its stage of development and possibly, the biochemical characteristics of the plant species [24]. While some studies have shown that the effects of tannins are due to their ability to interact and protect degradation of ruminal proteins [25], others have demonstrated direct toxic effects of tannins on nematodes [26] Findings from similar studies can therefore vary widely depending on the predominant mode of action. It has also been suggested that chemical structure of CT could be more important than their actual concentration as far as biological responses are concerned [27]. Results from the *in vitro* trials can be influenced and confounded by the presence, in the plants, of other unknown bioactive substances together with differences in nutritional values in addition of the different extraction solvents.

In the present study (Table 2), the egg inhibition capacity of different plants showed differences across the storage conditions and extraction solvents. This could be associated with concentration of condensed tannin in the plant species which has also affected by storage condition and extraction solvents. Molan *et al.* [28] confirmed the differences in the effectiveness of inhibiting egg hatchability by tannin extracts from different tanniferous plant species. Even though Albendazole, a

positive control, inhibited egg hatchability by 100%, it has clearly observed in the present study that the tannin extracts from various tannin rich plants had ability to inhibit egg hatchability to maximum 75.4%. This implies that effects of tannins, cheaper source from locally available plants, can be compared with artificial anthelmintics drugs. Tannins appear to reduce the hatching of fecal eggs [29]. The tannins could also bind with feed nutrients and possibly prevent bacterial growth in the faeces (larva feed on bacteria) and so limit the feed available for larval growth, or in some other way inhibit larvae growth and movement.

The highest larval development inhibition due to CTs extracted with 70% ethanol from *A. gummifera* in all storage conditions as compared to storage conditions of other plant species (Table 3) might be in conjunction with the concentration of tannin in each plants species and also the anthelmintic activity of tannins in plant extracts on larvae could be attributed to tannins capacity to bind to proteins and could operate via several mechanisms. Condensed tannins may bind to the cuticle of larvae, which is highly in glycoprotein and cause their death. *In vitro* experiments showed that prodelphinidin monomers and flavan-3-ol gallates were more active on the egg hatching, larvae motility and exsheathment of *H. contortus*, the procyanidins monomers and flavan-3-ol [30]. The tannins contained in the polar fraction of *Leuceana leucocephala* [31] and the flavonol glycosides in *sainfoin* (*Onobrychis viciifolia)* [13] have been demonstrated to have effects on the third stage larvae (L_3) of *H. contortus*.

In general, larval inhibition consecutively associated with CT concentration (Table 4). Molan *et al*. [28] reported the reduced the development of L_1 larvae to L_3 larvae and decreased the motility of L_3 larvae when assessed by the larval migration inhibition assay and this may reduce their infective capacity condensed tannins. In the present study, the anti-larval activity of tannin extracts were popular at fresh condition which disagrees with the works of Hoste *et al.* [24] that the most consistent results of anti-larval development activities were found with the plant extracts possessing the highest tannin content of various tanniferous plants.

Albendazole acts as cholinergic agonist on neuromuscular junctions in nematode parasites, which causes paralysis of the worm, leading to their death or expels them from the host. The differences in the extraction solvent might be associated with their chemical structure. A study reported by Eguale *et al*. [4] has shown that this route is predominant for the uptake of major broad spectrum anthelmintics by different nematode, cestode and trematode parasites as opposed to oral ingestion. This might be the direct or indirect effect of CTs some researchers believe that the plant tannins may affect parasites either directly or indirectly (or both). Tannins may react directly with adult worms by attaching to their "skin", causing them distress, or in directly by improving protein nutrition of the host and boosting the immune system [28].

The adult motility (Table 6) rate was associated with the concentration of CT in various storage conditions and plant species thus; tannin rich plants may represent a possible alternative option to control nematodes such as *H. contotus*. Max *et al*. [26], Mihreteab *et al.* [32] and Verma *et al.* [33] confirmed similar impression that anthelmintic activity of CTs in parasite reductions can have practical epidemiological implications in reducing pasture larval contamination.

In conclusion, the reduction in *H. contortus* egg hatchability, larva development and adult motility was clearly linked to CT concentration. More investigation is required by using a serial dilution test in further to allocate the effective and efficient dose. In spite of the advantages of *in vitro* tests on evaluation of anthelmintic activity of tannin rich plants, considerations should be taken in mind that potential bioactive substances used *in vitro* may not always correspond to *in vivo* bioavailability. Therefore, *in vitro* assays should always be accompanied by *in vivo* trials when used to validate anthelmintic activity of plant remedies.

ACKNOWLEDGEMENTS

Authors wish to thank the VLIR-UOS Institutional University Cooperation project (IUC-JU) for the financial support.

REFERENCES

1. Abunna, F., E. Tsedeke, B. Kumsa, B. Megersa, A. Regassa and E. Debela, 2009. Abomasal Nematodes: Prevalence in Small Ruminants Slaughtered at Bishooftu Town, Ethiopia. The Internet J. Vet. Med., 7(1). http://archive.ispub. com/journal/the-internet-journal-of-veterinary-medicine.
2. Almaz Habtamu and S.W. Mariam, 2011. Repeated simple sedimentation technique and prevalence of bovine schistosomosis in selected sites of Bahir Dar woreda. Ethiop. Vet. J., 15(1): 49-57.

3. Sissay, M.M., A. Uggla and P.J. Waller, 2007. Prevalence and seasonal incidence of nematode parasites and fluke infections of sheep and goats in eastern Ethiopia. Trop Anim Health Prod., 39(7): 521-31.
4. Eguale, T., G. Tilahun, A. Debella, A. Fleke and E. Makonnen, 2007. *Haemonchus contortus*: *in vitro* and *in vivo* anthelmintic activity of aqueous and hydro-alcoholic extracts of *Hedera Helix*. Experim. Parasitology, 116: 340-345.
5. Troell, K., P. Waller and J. Hoglund, 2005. The development and overwintering survival of free-living larvae of *Haemonchus contortus* in Sweden. J. Helminthology, 79: 373-379.
6. Athanasiadou, S., J. Githiori and I. Kyriazakis, 2007. Medicinal plants for helminth parasite control: facts and fictions. Animals, 1(9): 1392-1400.
7. Bizimenyera, E.S., J.B. Githiori, J.N. Eloff and G.E. Swan, 2006. *In vitro* activity of *Peltophorum africanum* Sond (Fabaceae) extracts on the egg hatching and larval development of the parasitic nematode *Trichostrongylus colubriformis*. Vet. Parasitology, 142: 336-343.
8. Pessoa, L.M., S.M. Morais, C.M.L. Bevilaqua and J.H.S. Luciano, 2002. Anthelmintic activity of essential oil of *Ocimum gratissimum* Linn. and eugenol against *Haemonchus contortus*. Vet. Parasitology, 109: 59-63.
9. Artho, R., M. Schnyder, L. Kohler, P.R. Torgerson and R. Hertzberg, 2007. Ivermectin resistance in gastrointestinal nematodes of Boer goats and Dorper sheep in Switzerland. Vet. Parasitol., 144: 64-73.
10. Bartley, D., E. Jackson, K. Jackson, R.L. Coop, B.B. Mitchell, J. Sales and F. Jackson, 2003. A survey of anthelmintic resistant nematode parasites in Scottish sheep flocks. Vet. Parasitology, 117: 61-71.
11. Chartier, C., F. Soubirac, I. Pors, A. Silvestre, J. Hubert, C. Couquet and J. Cabaret, 2001. Prevalence of anthelmintic resistance in gastrointestinal nematodes of dairy goats under extensive management conditions *in* southwestern France. J. Helminthology, 75: 325-330.
12. Alawa, C.B.I., A.M. Adamu, J.O. Gefu, O.J. Ajanusi, P.A. Abdu, N.P Chiezey, J.N. Alawa and D.D. Bowman, 2003. *In vitro* screening of two Nigerian medicinal plants (*Vernonia amygdalina* and *Annona senegalensis*) for anthelmintic activity. Vet. Parasitol., 113: 73-81.
13. Barrau, E., N. Fabre, I. Fouraste and H. Hoste, 2005. Effect of bioactive compounds from sainfoin (*Onobry -chis viciifolia* Scop.) on the *in vitro* larval migration of *Haemonchus contortus*: role of tannins and flavonol glycosides. Parasitology, 131: 531-538.
14. Yisehak, K. and Geert P.J. Janssens, 2013. Evaluation of nutritive value of leaves of tropical tanniferous trees and shrubs. Livestock Research for Rural Development, 25(2).
15. IAE/FAO, 2000. Quantification of Tannins in Tree Foliage IAEA, Vienna, Austria.
16. Baerman, G. and R. Wetzal, 1953. Cited after Soulsby E.J.L., 1965. Text book of veterinary clinical parasitology. Vol. I. Helminths, 1st Ed.Oxford. Black well Scientific Publications.
17. Jabbar, A., J. Iqbal, D. Kerboeuf, G. Muhammed, M.N. Khan and M. Afaq, 2006. Anthelmintic resistance: the state of play revisited. Life Science, 79: 2413-2431.
18. Coles, G.C., F. Jackson, W.E. Pomroy, R.K. Prichard, Samson-Himmelstjerna, G. von, Silvestere, A.M.A. Taylor and J. Vercruysse, 2006. The detection of Anthelmintic resistance In nematodes of Veterinary importance: Review. Vet. Parasitol., 136: 167-185.
19. Taylor, M.A., K.R. Hunt and K.L. Goodyear, 2002. Anthelmintic resistance detection methods. Vet. Parasitology, 103: 183-194.
20. Costa, C.T.C., C.M. Bevilaqua, A.L.F. Camurca-Vasconcelos, M.V. Maciel, S.M. Morais, C.M.S. Castro, R.R. Braga and L.M.B. Oliveira, 2008. *In vitro* ovicidal and larvicidal activity of Azadirachta indica extracts on *Haemonchus contortus*. Small Ruminants Res., 74: 284-287.
21. Peterson, D.G., K.S. Boehm and S.M. Stack, 1997. Isolation of milligram quantities of nuclear DNA from tomato (*Lycopersicon esculentum*), a plant containing high levels of polyphenolic compounds. Plant Mol. Biol. Rep., 15: 148-153.
22. SAS (statistical analysis system), 2010. Statistical analysis system (SAS/STAT program, version 9.3). SAS institute Inc, carry, NC, USA.
23. Brunet, S., F. Jackson and H. Hoste, 2008. Effects of sainfoin (*Onobrychis viciifolia*) extract and monomers of condensed tannins on the association of abomasal nematode larvae with fundic explants. International J. Parasitol., 38: 783-790.

24. Hoste, H., S. Brunet, V. Paolini, D. Bahuaud, S. Chauveau, I. Fouraste and Y. Le Frileux, 2009. Compared *in vitro* anthelmintic effects of eight tannin-rich plants browsed by goats in the southern part of France. Options Méditerranéennes, 85: 431-436.
25. Niezen, J.H., H.A. Robertson, G.C. Waghorn and W.A.G. Charleston, 1998. Production, Faecal egg counts and worm burdens of ewe lambs which grazed six contrasting forages. Vet. Parasitology, 80: 15-27.
26. Max, R.A., A.E. Kimambo, A.A. Kassuku, L.A. Mtenga and P.J. Buttery, 2007. Effect of tanniniferous browse meal on nematode faecal egg counts and internal parasite burdens in sheep and goats. South African J. Animal Science 2007, 37(2).
27. McNabb, W.C., J.S. Peters, L.Y. Foo, G.C. Waghorn and S.J. Jackson, 1998. Effect of condensed tannins prepared from several forages on the *in vitro* precipitation ofribulose-1,5-bisphospathe carboxilase (rubisco) protein and its digestion by trypsin (EC 2.4.21.4) and chymotrypsin (EC 2.4.21.1). J. Science Food and Agri., 77: 201-212.
28. Molan, A.L., R.A. Alexander, I.M. Brookes and W.C. McNabb, 2000. Effects of an extract from sulla (*Hedysarum coronarium*) containing condensed tannins on the migration of three sheep gastrointestinal nematodes *in vitro*. Proceedings of the New Zealand Society of Animal Production, 60: 21-25.
29. Min, B.R., S.P. Hart, D. Miller, G.M. Tomita, E. Loetz and T. Sahlu, 2005. The effect of grazing forage containing condensed tannins on gastrointestinal parasite infection and milk composition in Angora does. Vet. Parasitology, 130: 105-113.
30. Min, B.R., D. Miller, S.P. Hart, G. Tomita, E. Loetz and T. Sahlu, 2003. Direct effects of condensed tannins on gastrointestinal nematodes in grazing Angora goats. J. Animal Sci., 81(2): 23.
31. Ademola, I.O. and S.O. Idowu, 2006. Anthelmintic activity of *Leucaena leucocephala* seed extract on *Haemonchus contortus*-infective xlarvae. Vet. Rec., 158: 485-486.
32. Mihreteab, B., G. Tilahun, K. Yisehak and A. Mesele, 2011. *In-vitro* anthelmintic activity of condensed tannins from *Rhus glutinosa*, *Syzygium guineenses* and *Albizia gummifera* against sheep *Haemonchus contortus*. Global Veterinaria, 6(5): 476-484.
33. Vinod K. Verma, Khomendra Sarwa and Atul Kumar, 2013. Anthelmintic Activity of Fruit Peel and Root Extracts of *Trapa natans* L. var. *bispinosa* Roxb. Academic Journal of Plant Sciences, 6(2): 73-76.