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In vitro evaluation of anthelmintic activity of tannin-containing plant *Artemisia* extracts against *Haemonchus contortus* from goat

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ABSTRACT

Gastrointestinal nematode causes significant production losses in grazing ruminants and anthelmintic resistance are increasing at an alarming rate. The aim of this study was to evaluate the *in vitro* anthelmintic effects of tannin-containing plant *Artemisia vulgaris* against stomach worm *Haemonchus contortus* of goat. In this respect, *in vitro* anthelmintic activities of crude aqueous and ethanolic extracts of aerial parts of *A. vulgaris* were investigated on eggs, larvae (L3) and adults of *H. contortus* in the laboratory. Experimental plant *Artemisia* was collected, dried in shade and grinded to coarse powder and subjected to prepare crude aqueous and ethanolic extract. The experiment revealed that crude ethanolic extracts completely inhibited egg hatching at concentration of 5 mg/ml whereas aqueous extracts at concentration of 10 mg/ml. After exposure of 8 hours, the crude ethanolic extracts inhibited 69.33%, 51% and 38% larval motility tested at 20 mg/ml, 10 mg/ml and 5 mg/ml concentrations, respectively while the crude aqueousextracts inhibited 57.33%, 48.67% and 34.67% at the same concentrations. The ethanolic extract showed better *in vitro* effects against adult stomach worm than the aqueous extract in terms of motility inhibition at different hours post-treatment. Dose dependent effects were also observed for both extract. After 2 and 8 hours of exposure, the ethanolic extract induced 53.33% and 100% mortality at the highest tested concentration respectively, while the aqueous extract induced 47.67% and 86.67% at the same concentration. To our best knowledge, this is the first study in Bangladesh on the *in vitro* anthelmintic effects of *A. vulgaris* against *H. contortus.* Further *in vitro* and *in vivo* trials with this plant are required to evaluate their anthelmintic effects precisely.

Introduction

Gastrointestinal parasitism has been identified as one of the major constraints that hinder the development of livestock population (Kakar and Kakarsulemankhel, 2008) and adversely affects the health and productivity of animals. Despite significant losses by gastrointestinal parasitism, the problems are often neglected and overlooked as majority of the infected animals show a number of obvious clinical signs throughout their productive life and their effects are gradual and chronic (Raza *et al.,* 2010). Among the parasitic diseases, gastrointestinal nematodes such as *Haemonchus contortus*, *Trichostrongylus* spp., *Cooperia* spp., *Oesophagostomum columbianum*, *Trichuris* spp. and *Strongyloides papillosus* are most common in Bangladesh (Rahman and Mondal, 1983). The overall prevalence of gastrointestinal helminths in goat is 63.41% in Bangladesh (Hassan *et al.,* 2011). Overall 65.63% goats are infected with *H. contortus* in Bangladesh (Shahiduzzaman *et al.,* 2003). Grazing animals are most susceptible to gastrointestinal nematodes (GINs) infection than stall fed animals. GINs cause significant production loss in grazing ruminants. Adult stomach worm, *H. contortus* is a blood-sucking abomasal helminth of small ruminants responsible for major economic losses to producers worldwide. Furthermore, frequent uses of anthelmintics are increasing resistance at an alarming rate (Dyary, 2016).

In several developing countries, small farmers have limited access to commercial anthelmintics and veterinary services due to their non-availability and/or to their high cost. These stockowners rely on the ethno-

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veterinary medicine as an alternative and a sustainable control option readily adaptable (Hussain *et al.,* 2008; Sindhu *et al.,* 2010). For these various reasons, interest in the screening of medicinal plants for their anthelmintic activity remains of great scientific interest despite the extensive use of synthetic chemicals in modern clinical practices all over the world (Akhtar *et al.,* 2000). The Indian subcontinent mainly Bangladesh, Pakistan and India bound with plants that were known to have medicinal properties and have been used for their curative powers throughout the ages. Recently, much interest in the field of medicinal plants has started throughout the world and many countries have already come to realize not only potential as means of alleviating health problems but also their economic value (de Padua and Pancho, 1983).

The condensed tannin (CT) is a diverse group of polymeric flavonoids of tanniniferous plant origin that readily form complex with carbohydrates and improve metabolism in ruminants. Dietary supplementation of CT through forages, tropical tanniniferous tree leaves, skin and fruit have been found effective against different developmental stages of GIN parasites. *H. contortus* species was used by several scientists to evaluate the anthelmintic effects of various medicinal plant species (Alawa *et al.,* 2003; Assis *et al.,* 2003; Eguale *et al.,* 2007). *In vitro* assays are relevant and cheaper than *in vivo* methods. Egg hatch assay (EHA) is currently used for the detection of anthelmintic resistance in GIN (Timothy *et al.,* 2012). Suitable environment is required to perform *in vitro* assays of egg hatching, larval and adult worm motility of GINs. Higher concentration of GIN larvae occur at the temperature between 20 and 30ºC and rainfall between 31.9 and 122.7 mm. (Shahiduzzaman *et al.,* 1999).

There are a number of research works on the anthelmintic effects of medicinal plants in Bangladesh (Mostofa *et al.,* 1983; Begum *et al.,* 1997, Shahiduzzaman *et al.,* 2005). In this context, investigations on tannin-containing plants such as *Artemisia* species (Ashok and Kumud, 2010), *Terminalia arjuna, Fumaria perviflora, Moringa oleifera, Butea monosperma,* etc (Bauri *et al.,* 2015) might contribute to develop effective but low-cost herbal anthelmintic. However, these are mostly used in crude forms and their pharmacological preparations, dosages and mode of actions are not based on strong scientific evidence. Tannin-containing plant *A. vulgaris* is very much available in our country in and around the crop field, road side, bush, forest etc. There is a great opportunity to utilize this plant to minimize stomach worm of ruminants in Bangladesh. To our best knowledge, there is no published article on the anthelmintic activities of tannin-containing plants against stomach worm in Bangladesh. Therefore, this research work may provide useful information on anthelmintic activities of tannin containing plant *Artemisia* to use against stomach worm of ruminants

Materials and Methods

Collection and processing of plant Artemisia

The aerial part of the tannin-containing plant *Artemisia vulgaris* was collected from BAU campus and its surrounding rural areas and brought to the laboratory of the Department of Parasitology. After collection, the plant materials were rinsed under running tap water and cut into small pieces. Firstly the plant materials were dried in shade and then they were dried in the oven at 55-60°C until gain a constant weight.

Preparation of dust, crude extract and stock solution

Dust was prepared by pulverizing the dried plant materials and preserved these into an airtight plastic container till their use in extract preparation. Prepared plant dusts were used for preparation of plant extract and finally condensed extracts those were preserved in tightly corked-labeled bottle and stored in refrigerator until use (Akkari *et al.,* 2014). Stock solutions of plant extracts were prepared by diluting the condensed extracts with (PBS). Different concentrations i.e. 100 mg/ml, 50 mg/ml, 25 mg/ml of each category of plant extracts were prepared by dissolving them in the PBS prior to anthelmintic screening to use at concentrations 20 mg/ml, 10 mg/ml, 5 mg/ml, respectively.

Collection of adult worms, eggs and cultivation of larvae (L3) and their maintenance

The abomasum, small and large intestines of goats slaughtered in the local markets were collected and brought to the laboratory. They were opened in a plastic bucket separately and the contents were washed in tap water. The process was repeated for several times until the sediment becoming transparent. Then the adult stomach worms were collected in a petridish containing PBS and then kept in incubator at 38°C until required for experiment on the same day. The collected worms were washed for several times with PBS. Then uteri of gravid females were dissected out, crushed gently in a petridish. As a result eggs were released in the PBS solution. An *in vitro* culture system has already been established in our laboratory for harvesting the L_3 stage larvae of GINs (Shahiduzzaman *et al.,* 1999). A required volume of PBS was added to eggs and incubated at room temperature (25-30°C) for about 72 hrs transferred to a 100 ml beaker and incubated further until development of L_3. During cultivation the culture media were monitored every morning for observing the development. After development of L_3, the culture media were washed several times in PBS through centrifugation at 2000 rpm for 7 minutes and finally counted and suspended in a 100 ml beaker. After cultivation, the infective larvae were maintained in the laboratory by incubating them at 25-30°C and in sterile condition.

In vitro test of crude aqueous and ethanolic extracts for anthelmintic activity

Examination of eggs for development of larvae

Different concentration of crude aqueous and ethanolic extracts of *Artemisia* were used for treatment. Three concentrations (100 mg/ml, 50 mg/ml, and 25 mg/ml) for each extracts (stock solution) were used with three replicates of each. Untreated eggs in PBS with 0.5% Dimethyl sulf-oxide (DMSO) solution were used as negative control with three replicates. For aqueous extracts, every 1 ml stock solution was placed in each test tube containing approximately, 200 eggs in 4 ml of PBS. After placing stock solution, each tube was then contained 5 ml with the concentration of 20mg/ml, 10mg/ml, and 5mg/ml of aqueous extracts respectively. For ethanolic extracts, at the same concentrations, 1 ml stock solution in 4 ml PBS with 0.5% DMSO was used. The test tubes were covered and incubated at room temperature (25-30°C) for 72 hours. Hatched larvae (dead or alive) and unhatched eggs were counted under dissecting microscope at 40*x* magnification and calculated (Akkari *et al.*, 2014).

Detection of larval motility

Untreated Larvae in PBS with 0.5% DMSO solution were used as negative control with three replicates. For aqueous extracts, every 1 ml stock solution was placed in each petridish containing 100 larvae (L_3) in 4 ml of PBS. Each petridish was then contained 5 ml with the concentration of 20mg/ml, 10mg/ml, and 5mg/ml of aqueous extracts respectively. For ethanolic extracts, at the same concentrations, 1 ml stock solution in 4 ml PBS with DMSO (0.5%) was used. All the tests were performed in triplicates. The petridishes were covered and incubated at room temperature (25-30°C). Inhibition of larval motility was the criteria of anthelmintic activity. The required delays for larvae paralysis and/or complete immobility were recorded at 0; 2; 4; 8; 16 and 24 hours. To test if the larvae could retrieve their motility after 24 h, they were washed with distilled water and resuspended in PBS for 30 min. Worms death was ascertained by the absence of motility during an observation period of 5–6 sec. Motile (live) or non-motile (dead) larvae (L_3) were counted under dissecting microscope at 40*x* magnification and calculated (Akkari *et al*., 2014).

Detection of adult worm's motility

Untreated Adults in PBS with 0.5% DMSO solution were used as negative control with three replicates. Similarly, for aqueous extracts, every 1 ml stock solution was placed in each petridish containing 5 adult worms in 4 ml of PBS with same concentration. An exactly similar protocol was applied to test L_3 stage and adult worm's motility for ethanolic extracts (Akkari *et al.*, 2014).

Statistical analysis

In vitro effects of different concentration of *Artemisia* extract were statistically analyzed with ANOVA technique to obtain the level of significance using MSTAT-C package program developed by Russel (1986). The mean differences were compared by Duncan's New Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

Results and Discussion

Egg hatch assay

In this study, ethanolic extracts showed higher inhibitory effects than aqueous extract on egg hatching. In ethanolic extracts, none of the eggs were hatched at each of the 5 mg/ml, 10 mg/ml and 20 mg/ml concentration whereas in aqueous extracts, none of the eggs were hatched at the concentration of 20 mg/ml and 10 mg/ml but 25% eggs were hatched at the concentration of 5 mg/ml. The 5 mg/ml crude ethanolic extracts completely (100%) inhibited egg hatching whereas10 mg/ml of crude aqueous extracts (Table 1) did. These findings could also be compared with Akkari *et al.* (2014) who recorded the findings that the maximum concentration required to induce complete (100%) egg hatch inhibition for crude aqueous and ethanolic extracts was 2 mg/ml. *Thymus capitatus* (another important pastoral species form arid Tunisia) aqueous and ethanolic extracts totally inhibited *H. contortus* egg hatching at 2 mg/ml concentration (Landolsi *et al*., 2013).

Larval motility assay

This study revealed that inhibition of larval motility increased significantly with the concentration of the extract dose of *A. vulgaris*. The crude ethanolic extracts inhibited the motility of 35.33%, 44.33%, 69.33% and 100% *Haemonchus* larvae after exposure to 2, 4, 8 and 16 hours respectively at 20 mg/ml concentrations whereas motility of 19.67%, 34.33%, 51% and 100% larvae were inhibited at 10 mg/ml concentrations and motility of 11.33%, 17.33%, 38% and 66.33% larvae were inhibited at 5 mg/ml concentrations after exposure to 2, 4, 8 and 16 hours respectively (Table 2). The crude aqueous extracts inhibited the motility of 27.67%, 33%, 57.33%, 87.33% and 100% *Haemonchus* larvae after exposure to 2, 4, 8, 16 and 24 hours respectively at 20 mg/ml concentrations whereas motility of 13.67%, 23.67, 48.67%, 69.67% and 78.67% larvae were inhibited at 10 mg/ml concentrations and 0%, 17%, 34.67%, 62.33% and 67.33% at 5 mg/ml concentrations after exposure to 2, 4, 8, 16 and 24 hours respectively (Table 3). Crude ethanolic extracts showed higher inhibitory effects than crude aqueous extract on the inhibition of larval motility at each concentration level.

Table 1. Hatchability rate (%) of *Haemonchus contortus* eggs after treatment with crude ethanolic and aqueous extracts

Extracts	Concentration (mg/ml)	Total eggs	Hatched eggs hatched	Hatchability rate (%)
Ethanolic	5	200	0	0
	10	200	0	0
	20	200	0	0
Aqueous	5	200	50	25
	10	200	0	0
	20	200	0	0
Negative Control	0	200	180	90

Table 2. Motility inhibition rate (%) of larvae (L3) of *Haemonchus contortus* post-exposure to crude ethanolic extract treatments

Concentration (mg/ml)	Motility inhibited (%)					
	0 hour	2 hours	4 hours	8 hours	16 hours	24 hours
5	0±0.00	11.33±7.220c	17.33±7.62c	38±5.72c	66.33±9.22b	100±0.99
10	0±0.00	19.67±6.42b	34.33±8.52b	51±4.02b	100±5.32a	-
20	0±0.00	35.33±10.12a	44.33±9.62a	69±12.02a	100±6.42a	-
Negative control	0±0.00	0±0.00d	0±0.00d	0±0.00d	0±0.00c	4.67±2.00
P- value		0.001	0.001	0.001	0.001	0.001
Sig. level		**	**	**	**	NS

Table 3. Motility inhibition rate (%) of larvae (L3) *Haemonchus contortus* post-exposure to crude aqueous extract treatments

Concentration (mg/ml)	Motility inhibited (%)					
	0 hour	2 hours	4 hours	8 hours	16 hours	24 hours
5	0±0.00	0±0.00c	17±10.72c	34.67±12.47c	62.33±15.72c	67.33±10.22c
10	0±0.00	13.67±7.52b	23.67±9.44b	48.67±10.52b	69.67±16.72b	78.67±9.52b
20	0±0.00	27.67±6.63a	33±8.99a	57.33±11.22a	87.33±12.12a	100±17.92a
Negative control	0±0.00	0±0.00c	0±0.00d	0±0.00d	0±0.00d	4.67±0.10d
P- value		0.001	0.001	0.001	0.001	0.001
Sig. level		**	**	**	**	**

Table 4. Mortality rate (%) of adult *Haemonchus contortus* worms after exposure to crude ethanolic extract treatments

Concentration (mg/ml)	Mortality rate (%)					
	0 hour	2 hours	4 hours	8 hours	16 hours	24 hours
5	0±0.00	33.33±0.06b	53.33±0.09b	73.33±0.11b	93.33±9.43	100±0.00
10	0±0.00	46.67±0.05b	60.00±0.08ab	86.67±0.12ab	100±0.00	-
20	0±0.00	53.33±0.07a	73.33±0.06a	100±0.10a	-	-
Negative control	0±0.00	00±0.00c	00±0.00c	00±0.00c	00±0.00b	6.67±0.00
P- value		0.001	0.001	0.001	0.001	
Sig. level		**	**	**	**	

Table 5. Mortality rate (%) of adult *Haemonchus contortus* worms after exposure to crude aqueous extract treatments

Concentration (mg/ml)	Mortality rate (%)					
	0 hour	2 hours	4 hours	8 hours	16 hours	24 hours
5	0±0.00	26.67±0.06b	40±0.10b	46.67±0.10c	73.33±0.12b	93.33±0.04a
10	0±0.00	33.33±0.07b	53.33±0.11ab	73.33±0.11b	93.33±0.10a	100±0.03a
20	0±0.00	46.67±0.08a	60.00±0.10a	86.67±0.08a	100±0.10a	-
Negative control	0±0.00	0±0.00c	0±0.00c	0±0.00d	0±0.00c	6.67±0.02b
P- value		0.001	0.001	0.001	0.001	0.001
Sig. level		**	**	**	**	**

** = Significant at 1% level of probability ($p<0.01$); NS = Not significant ($p>0.05$); In a column figures with same letter or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT)

Adult worm's mortality assay

In case of adult worm's mortality assay, after 2, 4, and 8 hours of exposure, the crude ethanolic extract induced 53.33%, 73.33% and 100% mortality at tested 20 mg/ml concentration respectively, (Table 4) while the crude aqueous extract induced 47.67%, 60% and 86.67% at the same concentration, respectively (Table 5). These findings could also be compared with Akkari *et al.* (2014) who recorded the findings of *Artemisia campestris* crude extracts against *H. contortus* that induced 91.3% and 100% mortality at the tested concentration of 2 mg/ml after 8 and 24 h of exposure of the crude ethanolic extract, respectively, whereas the crude aqueous extract induced 3.22% and 70.96% at the same concentration, respectively.

This study revealed that all three different concentrations of crude ethanolic and aqueous extracts have *in vitro* anthelmintic effects against eggs, larvae and adult *H. contortus*. However, *in vitro* anthelmintic effects of several plants have been demonstrated against stomach worm of goats in various parts of the world (Akhtar *et al.*, 2000; Dano and Bogh, 1999). *In vitro* screening of both crude aqueous and ethanolic extract are more or less similar but ethanolic extract showed more anthelmintic activity against both larvae and adult worms (Sisay *et al.*, 2012).

In this study, it was found that in all cases, the complete inhibition of egg hatching, larval motility and adult worm mortality depends on the type and concentration of the extracts used (Tables 1, 2, 3, 4 & 5). The time for complete inhibition in motility of larvae and mortality of adult decreased as the concentrations of these crude extracts increased (Table 2, 3, 4 & 5). This observation indicated that mortality rate varied significantly among the plants, solvents and doses. So, it is clear from the results of *in vitro* efficacy test that, *Artemisia* plants, as its crude aqueous and ethanolic extracts form, had high anthelmintic effects on eggs, larvae and adult *H. contortus* from goats *in vitro*. This was also indicated by a number of previous researchers (Akkari *et al.*, 2014; Cala *et al.*, 2014; Ahmed *et al.*, 2011; Squires *et al.*, 2011; Hart *et al.*, 2008; Eguale *et al.*, 2007).

From the study, it was found that *A. vulgaris* crude aqueous and ethanolic extracts showed significant ($p<0.01$) *in vitro* anthelmintic activity against *H. contortus* of goat as ascertained by egg hatching inhibition and larvae and adult worm motility inhibition. In addition, mortality was higher with the ethanolic extract treatment, whereas the worms survived for a significantly longer period in the presence of aqueous extract. This indicated that ethanolic extract has greater anthelmintic activity than the aqueous extract.

Conclusion

The present study evaluated the *in vitro* effects of the plant *Artemisia* against the eggs, larvae and adult *H. contortus*. Further *in vitro* and *in vivo* trials at different concentrations against different worm species and stages are required to determine a more precise anthelmintic activity of this plant species and its potential use in controlling small ruminant's gastrointestinal nematodes.

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References

Ahmed, A., Lidia, A.G., Hajer, E.J. and Pablo, C.M. 2011. Antioxidant and antitumor activities of *Artemisia campestris* and *Thymela ahirsuta* from southern Tunisia. *Food and Chemical Toxicology*, 49: 342–347. https://doi.org/10.1016/j.fct.2010.11.003

Akhtar, M.S., Iqbal, Z., Khan, M.N. and Lateef, M. 2000. Anthelmintic activity of medicinal plants with particular reference to their use in animals in the Indo Pakistan Subcontinent. *Small Ruminant Research*, 38: 99–107. https://doi.org/10.1016/S0921-4488(00)00163-2

Akkari, H., Rtibi, K., Bchir, F., Rekik, M., Darghouth, M.A. and Gharbi, M. 2014. *In vitro* evidence that the pastoral *Artemisia campestris* species exerts an anthelmintic effect on *Haemonchus contortus* from sheep. *Veterinary Research Communications*, 123: 112–126.

Alawa, C.B., Adamu, A.M., Getu, J.O., Ajansui, O.J., Abdu, P.A., Chiezey, N.P., Alawa, J.N. and Bowman, D.D. 2003. *In vitro* screening of two Nigeria medicinal plants (*Vernonia amiygdalina* and *Annona senegalensis*) for anthelmintic activity. *Veterinary Parasitology*, 113: 73–81. https://doi.org/10.1016/S0304-4017(03)00040-2

Ashok, P.K. and Kumud, U. 2010. Preliminary phytochemical screening and physico-chemical parameters of aerial parts of *Artemisia vulgaris*. *International Journal of Research in Ayurveda and Pharmacy*, 1(1): 206–211.

Assis, L.M., Bevilaqua, C.M.L., Morais, S.M., Vieira, L.S., Costa, T.C. and Souza, A.L. 2003. Ovicidal and larvicidal activity *in vitro* of *Spigelia anthelmia Linn* extracts on *Haemonchus contortus*. *Veterinary Parasitology*, 117: 43–49. https://doi.org/10.1016/j.vetpar.2003.07.021

Bauri, R.K., Tigga, M.N. and Kullu, S.S. 2015. A review on use of medicinal plants to control parasites. *Indian Journal of Natural Products and Resources*, 6(4): 268–277.

Begum, T. 1997. Comparative efficacy of some indigenous plants (Bironja, Turmeric and Veranda) with that of patent drug Nilzan against nematodiasis in sheep. *Thesis, M. S. in Pharmacology*, Bangladesh Agricultural University, Mymensingh.

Cala, A.C., Ferreira, J.F.S., Chagas, A.C.S., Gonzalez, J.M., Rodrigues, R.A.F., Foglio, M.A., Oliveira, M.C.S., Sousa, I.M.O., Magalhães, P.M. and Junior, W.B. 2014. Anthelmintic activity of *Artemisia annua L.* extracts *in vitro* and the effect of an aqueous extract and artemisinin in sheep naturally infected with gastrointestinal nematodes. *Parasitology Research*, 113: 2345–2353. https://doi.org/10.1007/s00436-014-3891-z

Dano, A.R. and Bogh, H.O. 1999. Use of herbal medicine against helminth in livestock, renaissance of an old tradition. In World Animal Review, (the FAO). *Journal of Animal Health Production and Products*, 2: 60–68.

de Pauda, L.S. and Pancho, V. 1983. Handbook on Philippine Medicinal Plants. *Vol. 1. Documentation and Information Research*, UPLB. P.24.

Dyary, H.O. 2016. Veterinary anthelmintics and anthelmintic drug resistance. *Journal of Zankoy Sulaimani*, 18(1): 91–206. https://doi.org/10.17656/jzs.10463

Eguale, T., Tilahun, G., Debella, A., Feleke, A. and Makkonen, E. 2007. *Haemonchus contortus: in vitro* and *in vivo* anthelmintic activity of aqueous and hydro-alcoholic extracts of *Hedera helix*. *Experimental Parasitology*, 52: 99–109. https://doi.org/10.1016/j.exppara.2007.01.019

Gomez, K.A. and Gomez, A. A. 1984. Statistical Procedures for Agricultural Research. *A book of International Rice Research Institute*, 2nd edition.

Hart, S.P., Ferreira, J.F.S. and Wang, Z. 2008. Efficacy of wormwoods (*Artemisia* spp.) as an anthelmintic in goats. *Journal Animal Science*, 86: 92.

Hassan, M.M., Hoque, M.A., Islam, S.K.M.A., Khan, S.A., Roy, K. and Banu, Q. 2011. A prevalence of parasites in Black Bengal goats in Chittagong. *Bangladesh International Journal of Livestock Production*, 2: 40–42.

Hussain, A., Khan, M.N., Iqbal, Z. and Sajid, M.S. 2008. An account of the botanical anthelmintics used in traditional veterinary practices in Sahiwal district of Punjab. *Pakistan Journal of Ethnopharmacology*, 119: 185–190. https://doi.org/10.1016/j.jep.2008.06.034

Kakar, M.N. and Kakarsulemankhel, J.K. 2008. Prevalence of endo (trematodes) and ecto-parasites in cows and buffaloes of Quetta, Pakistan. *Pakistan Veterinary Journal*, 28: 34-36.

Landolsi, M.R., Akkar, H., Bchir, F., Gharbi, M., Mhadhbi, M., Aouadi, S. and Darghouth, M.A. 2013. *Thymus capitatus* from Tunisian arid zone: chemical composition and *in vitro* anthelmintic effects on *Haemonchus contortus*. *Veterinary Parasitology*, 197: 374–378. https://doi.org/10.1016/j.vetpar.2013.05.016

Mostofa, M. 1983. Efficacy of some indigenous medicinal plants against gastro-intestinal nematodiasis in cattle and their comparative activity with that of nemafex. *Thesis, M. Sc. (Vet. Sc.) in Pharmacology*, Bangladesh Agricultural University, Mymensingh.

Rahman, M.H. and Mondal, M.M.H. 1983. Helminth parasites of cattle (*Bos tauras*) in Bangladesh. *Indian Journal of Parasitology*, 7: 173–174.

Raza, A.M., Murtaza, S., Bachaya, H.A., Qayyum, A. and Zaman, M.A. 2010. Point Prevalence of *Toxocara vitulorum* in large ruminants slaughtered at Multan abattoir. *Pakistan Veterinary Journal*, 30: 242–244.

Shahiduzzaman, A.K.M., Talukder, M.H. and Rahman, M.H. 1999. Ecology of preparasitic stages of *Strongyles* of ruminants in Bangladesh. *Bangladesh Veterinary Journal*, 33: 93–97. https://doi.org/10.3329/bjvm.v3i1.11355

Shahiduzzaman, A.K.M., Talukder, M.H. and Rahman, M.H. 1999. Laboratory studies on the development of preparasitic stages of *Haemonchus contortus* and *Oesphagostomum radiatum*. *Bangladesh Veterinary Journal*, 33: 103–106. https://doi.org/10.3329/bjvm.v1i1.1917

Shahiduzzaman, M., Islam, M.K., Rahman, M. and Mondal, M.M.H. 2005. *In vitro* effects of some household chemicals on infective larvae of *Haemonchus contortus* of goat. *Bangladesh Journal of Veterinary Medicine*, 3: 55–58.

Shahiduzzaman, M., Alim, M.A., Rahman, M. and Mondal, M.M.H. 2003. Seasonal influence on the occurance of *Haemonchus contortus* infection in slaughtered Black Bengal goats in Bangladesh. *Bangladesh Journal of Veterinary Medicine*, 1: 45–48.

Sindhu, Z.U.D., Iqbal, Z., Khan, M.N., Jonsson, N.N. and Siddique, M. 2010. Documentation of ethno-veterinary practices used for treatment of different ailments in selected a hilly area of Pakistan. *International Journal of Agricultural Biology*, 12: 353–358.

Sisay, G., Nuraddis, I., Belay, A. and Tadesse, E. 2012. *In vitro* Evaluation of Anthelmintic Activities of Crude Extracts of Selected Medicinal Plants Against *Haemonchus Contortus* in Alemgena, Ethiopia. *Acta Parasitologica Globalis* 3: 20–27.

Squires, J.M., Ferreira, J.F.S., Lindsay, D.S. and Zajac, A.M. 2011. Effects of artemisinin and Artemisia extracts on *Haemonchus contortus* in gerbils (*Meriones unguiculatus*). *Veterinary Parasitology*, 175: 103–108. https://doi.org/10.1016/j.vetpar.2010.09.011

Timothy, G., Barry, C.G., Philip, J.H., Skuce, G.S.H., Maeder, S., Holdsworth, P., Pomroy. W. and Vercruysse, J. 2012. World Association for the Advancement of Veterinary Parasitology (WAAVP) Anthelmintic products targeting nematode infections of ruminants and horses. *Veterinary Parasitology*, 190: 306–316. https://doi.org/10.1016/j.vetpar.2012.09.004