Egg Hatch Assay and Lavicidal Activity of *Piliostigma Thoningii* Pod Extract and Fractions on *Haemonchus contortus*

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SUMMARY

The inhibitory effects of *Piliostigma thoningii* pod extract and fractions were carried out on *Haemonchus contortus*, a gastrointestinal nematodes of small ruminants (sheep and goats). Egg hatching and larvicidal inhibitory effects of pod extract and fractions of *P. thonnigii* pods were determined using the egg inhibition assay (EIA) and larval development inhibition assay (LDIA) assay. The assay was tested in 96-flat bottom microtitre plate, using different concentrations of 0.78, 1.56, 3.125, 6.25 and 12.5 mg/ml of the experimental extracts with three replicates. The plant extract showed higher anthelmintic activity after exposing *H. Contortus* to the EIA and LDIA assays. It was observed that at 12.5 mg/ml, the crude extract and albendazole had 100% inhibitory effects on the ovicidal and larvicidal activities of the parasites, showing no significant difference ($p>0.05$) when compared, while n- butanol had significant ($p<0.05$) inhibitory effects on the hatching of eggs and n- butanol, ethyl acetate, petroleum ether and aqueous had significant ($p<0.05$) inhibitory effects on the larval development when compared with albendazole. However, the fractions tested on *H. Contortus* at the concentrations of 0.78, 1.56, 3.125 and 6.25 mg/ml possessed anthelmintic activity that produced above 90% ovicidal and larvicidal inhibition of the parasite. The anthelmintic effect of *P.thoningii* on *H. contortus* in this study is further subjected to confirmation through *In vivo* and toxicity profile studies.

Keywords: *Anthelmintic, P. thonningii, H. contortus, Pod, Fractions, Albendazole.*

INTRODUCTION

Globally, parasitic infections in ruminant production causes major health problem that severely limits the productivity of ruminants livestock by causing a debilitating impact on animal health (Jegede *et al*., 2013, Swarmakar *et al*., 2015). *Haemonchus contortus* is a parasitic gastrointestinal parasite of small ruminants such as Sheep and goats (Schoeman. 2003).

In Nigeria, the parasite is found in the tropical rainforest areas where the climates are humid and the environment favours the developmental cycle of the parasite (Ademola *et al*., 2005, Fentahun and Luke, 2012). *H. contortus* are predominantly found in the abomasums and mostly feeds on blood contents, their feeding activities in the abomasum causes anemia, diarrhea, weight loss, loss of condition, sometimes sudden death of infected animals, therefore, *H. contortus* is a parasitic disease of economical importance that causes great losses to livestock production (Nabi *et al*., 2014, Squires *et al*., 2011).

Heamonchosis is a clinical disease condition caused by *H. contortus* infection which gradually results into abnormal hematological changes, abnormal biochemical reactions and changes in values of the body parameters of the infected animal (Hassan *et al*., 2012, Ijaz *et al*., 2009).

Helminthosis in sheep and goats is a major parasitic gastrointestinal disease that affects small ruminant production globally; this disease condition is usually complicated by the indiscriminate use or inappropriate dosage of anthelmintics on the animals, when trying to control helminthes infections, thereby causing resistance of the nematodes species to conventional drugs (Aktar *et al*., 2000, Ferreira *et al*., 2013).

Anthelmintics derived from plant phyto-constituents, synthetic compounds with good grazing management and hygienic controls have been employed over the past years in the control of parasitic gastrointestinal nematodes of ruminants (Fentahun and Luke, 2012, Badar *et al*., 2011, Martins, 1997). Previous research has shown that herbs and plants extracts possess some inhibitory compounds and anthelmintic activities on gastrointestinal helminthes such as *H. contortus*.

Therefore, both *in vitro* and *in vivo* studies are required to explore the potentials of these plants and their folkloric claims (Biffa *et al*., 2004, Jegede *et al*., 2009, Maphosa *et al*., 2010).

Piliostigma thonningii is a leguminous and a perennial tree found in the tropical African and popularly called camel's foot, kamelspoor and molgoropo.

In the Western African countries particularly Nigeria, it is called, nyihar (Tiv), abafe (Yoruba), kalgo (Hausa), okpoatu (Igbo) andejei-jei (Igala). In some African countries the plant part such as the root, bark, pods and fruit are used for medicinal purpose (Silva *et al*., 1997).

The leaf and root preparations are used in the treatments of some viral infections such as hepatitis B and C, HIV virus, it is also used to facilitate wound healing and treat skin diseases.

Previous study reported the use of *P. thonningii* as anthelmintic, anti-inflammatory, analgesic, diarrheal and antibacterial in tropical Africa (Aderogba *et al*., 2006, Akinpelu *et al*., 2000, Lemessa, 2010). The stem bark of this plant has been used as anthelmintic against *Ascaridia galli* infected corckrels, against the Rat tapeworm *Hymenolepis diminuta* and the ethanolic extract of the *P. thonningii* bark D-3-O-methylchiroinositol showed anthelmintic activity against *H. contortus* larvae (Asuzu and Onu, 1994, Asuzu *et al*., 1999, Lemessa, 2010). The present study establishes the anthelmintic activities of *Piliostigma thonningii* pods on *Haemonchus contortus* using albendazole as the positive control.

MATERIAL AND METHODS

Study area

The study was carried out at the Department of Parasitology and Entomology (Helminthology Laboratory) Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Zaria has a coordinates of 11°04´N 7°42´E and a total land square meter of 563km² (217sq m) and it is located on the kubbani river (Tributary of the Kaduna) (Travelmath, 2011). Zaria has a tropical wet (April to September) and dry climate (October to March) seasons (Encyclo.Britannica, 2018).

Plant collection and extraction

Fresh pods of *P. thoningii* collected from Enugu state, Nigeria was identified by Mr. Alfred Ozioko (Taxonomist). The pods were washed, air- dried, pulverized and sieved. Three hundred grams of the pulverized plant sample material was weighed and extracted with 80% methanol in a Soxhlet apparatus. The extracted sample was concentrated using a rotary evaporator coupled to a thermo-regulator.

Solvent Partitioning

The extracted sample was suspended in distill water and subsequently partitioned with petroleum ether, ethyl acetate and n-butanol, using 150 ml of each solvent. The whole process was repeated in triplicates for each solvent (Simon *et al*., 2008, Olayemi *et al*., 2019).

Collection of parasites

The *H. contortus* parasites were obtained from the abomasums of infected sheep slaughtered at the Zaria abattoir. The parasites were recovered by washing the abomasal content through a sieve. Adult females of *H. contortus* were identified, individually picked using wire loop and crushed together with horse feaces. The mixture was then cultured in a clean glass jar at 27 °C. Modified Baerman's apparatus was used to harvest the larvae (L1) after 8 days (Simon *et al*., 2012, Olayemi *et al*., 2019).

In vitro egg hatch assay

The guidelines and procedure used for the egg Hatch Inhibition Assay (EHIA) in this study was described by World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles *et al*., 1992).

Adult female *H. contortus* parasites were identified and separated by method described by Taylor *et al*. (2007) and suspended in distilled water and were crushed to liberate the parasite eggs (Simon *et al*., 2012). Two hundred (200) eggs of *H. contortus* contained in 0.08ml was pipetted into a 96- flat bottomed micro titre plate in addition with 0.5ml at different concentration (0.78, 1.56, 3.125, 6.25 and 12.5 mg/ml) of the pod extract and the fractions. Similarly, albendazole at (0.5ml in each well) of different concentrations (0.78, 1.56, 3.125, 6.25 and 12.5 mg/ml) and distilled water (0.5ml in each well) were also tested.

The experiment was carried out in triplicates. The eggs were cultured for 48 hours at room temperature, after which a drop of lugol's iodine was added to inhibit further hatching. Thereafter, the content of each 96-well microtitre plate were examined (Olayemi *et al*., 2019).

Evaluation of larvicidal activity of the extract

The evaluation of the larvicidal activities of the extract and fractions was done according to methods described by Wabo *et al*. (2011). One hundred (100) larvae of *H. contortus* contained in 0.1 ml of distill water were added into each of a labelled 96 -flat-bottom micro titre plate and 0.5 ml of the different concentrations (0.78, 1.56, 3.125, 6.25 and 12.5 mg/ml) of the pod extract and fractions were added. Each test was done in triplicate.

The plates were covered with foil paper and left at room temperature for 24 hours. Thereafter, the contents of the pod extract, fractions and albendazole in the 96 flat-bottom microtitre plate were examined. The movements or migration of the larva from one point to the other was used to consider the parasites mortality.

Statistical analysis

The Mean (±SD) percentages inhibition at different concentrations with the controls were determined by one way ANOVA. The Post Hoc statistical significance test employed was Least square difference (LSD), while the difference between the means was considered significant at (P < 0. 0.5).

RESULTS

The phytochemical analysis shows the chemical compound (metabolites) contained in the *P. thoningii* pod crude extracts. It was observed that steroids and cardiac glycoside were not observed and tannins were present in the hydrolyzed form as shown in (Table 1).

The pod extract and fractions showed significant ($p < 0.05$) difference in the ovicidal and larvicidal inhibitory effects on *H. contortus* in graded doses.

There was a positive correlation between the concentrations of the crude extract, fraction, albendazole and the rates of egg hatch inhibition such that, as the drug concentration increases, the egg hatch and larval development inhibition rate increases (Tables 2, 3, and Supplementary Tables 4 and 5).

Although there were variations in concentrations (mg/ml) required for each of the pod extract and fractions to show individual anthelmintic activity and efficacy.

Table 1: Qualitative phytochemical analysis

Phytochemical analysis	Plant constituents	*P. thoningii* extract
Froting test	Saponin	+
Dragendoff test	Alkaloid	+
Molish test	Carbonhydrate	+
Leiberman Bucchard test	Steroid	-
Leiberman Bucchard test	Triterpine	+
Keller kiliani test	Cardiac glycoside	-
Ferric chloride test	Tannin	+ (H)
Sodium hydroxide	Flavonoid	+
Bontrager's test	Anthraquinone	+
Ferric chloride test	Phenol	+

(+) indicates the presence of the component, while (-) indicates the absence of the phytochemical compound in the extract. The plant extract had all the listed secondary metabolites. Tannins were present in the hydrolysed (H) form.

Table 2: Mean (±SD) percentage inhibition of egg hatch of *H. contortus* at different concentration of *P. thoningii* extract (mg/ml) and Albendazole (mg/ml).

Treatments	0.78mg/ml	1.56mg/ml	3.125mg/ml	6.25m/ml	12.50mg/ml
P. thoningii	95.17 ± 1.04^b	97 ± 1.32^b	98 ± 1.32^b	99.17 ± 1.04^b	100 ± 0.00^b
Albendazole	96.83 ± 1.04^b	98 ± 0.5^b	99 ± 0.00^b	99.83 ± 0.289^b	100 ± 0.00^b
DW	6.2 ± 2.33				

Means with different superscript letter (a, b, c) differ significantly ($p<0.05$) from the positive control group
DW: Distilled water

Table 3: Mean (±SD) percentage inhibition of larval development of *H. contortus* at different concentration of *P. thoningii* extract (mg/ml) and Albendazole (mg/ml).

Treatments	0.78mg/ml	1.56mg/ml	3.125mg/ml	6.25m/ml	12.50mg/ml
P. thoningii	71.3±3.06[a]	75±3.0[a]	82±2.0[a]	86±3.6[a]	99.3±0.28[b]
Albendazole	73.46±2.25[b]	77±1.73[b]	91.3±3.2[b]	100±0.00[b]	100±0.00[b]
DW	12. 3 ± 1.45				

Means with different superscript letter (a, b, c) differ significantly ($p<0.05$) from the positive control group
DW: Distilled water

This study showed that the pod extract and albendazole had 100% anthelmintic activity on *H. contortus* at the concentration of 12.5 mg/ml, showing no significant ($p>0.05$) difference in their inhibitory effects, while n- butanol showed a significant ($p<0.05$) inhibitory difference on the hatching of eggs and larvicidal inhibition by n- butanol, ethyl acetate, petroleum ether and aqueous when compared with albendazole. However, all the tested extracts produced over 50% ovicidal and larvicidal effects at different ranges of 0.78 – 12.5 mg/ml as observed in this study.

DISCUSSION

This study showed that, the increase in the mean percentage ovicidal and the larvicidal inhibitory effects is proportional to the increase in chemical ingredients, supplementary input of different active compound, potential effect and anthelmintic activity of the plant extracts.

Thus, a significant difference ($p< 0.05$) was observed with the anthelmintic activity at different drug concentrations (Dabella, 2002, Kipyegon, 2017).

In addition, the study shows that the inhibitory effects of the tested extracts, increases in a dose graded manner. All the extracts of *P. thoningii* pods showed significant inhibitory effect on hatching of the parasites eggs. The *P. thoningii* plant pods showed 100% inhibition of egg hatching and larval development which statistically ($p>0.05$) did not show significant ($p>0.05$) different from albendazole activity the positive control. Furthermore, the present study also showed that the larval mortality rates increases as the concentration level increases for all the tested extracts. The larvicidal effect of the fractions of *P. thoningii* pods on *H. contortus* larvae when compared with that of albendazole activity showed significant differences ($p<0.05$).

It was also observed that the egg hatch inhibitory activities were more effective with the crude extract than the fractions; this could be attributed to the penetration of the active phyto-constituents of the extracts into the parasite's egg shell, which affects the segmentation process of the eggs and inhibits the hatching of the eggs. However, it could also be as a result of the structural and different mechanism of absorption between the egg shell and cuticle of larvae of *H. contortus* (Eguale *et al*., 2006, Eneojo *et al*., 2015, Kipyegon, 2017, Kollins *et al*., 2012).

The findings on *P. thoningii* pods in this study was in agreement with previous study by Asuzu and Onu, (1994) and Asuzu *et al*. (1999) that established the anthelmintic effects of ethanolic extract and D-3-O-Methylchiroinositol of *P. thoningii* bark and stem bark respectively.

D-3-O-Methylchiroinositol is an isolate derived from alkaloids present in *P. thoningii* plants and this metabolite could be responsible for the inhibitory anthelmintic effects observed on the parasites. The study by Asuzu *et al*. (1999) showed that as the plant extracts concentration (mg/ml) increases, there was also an increase in the inhibitory anthelmintic effects on the neuromuscular junction and the ganglion of the parasites.

Previous studies had shown that plant extract usually produce concentration – dependent effects when tested on helminth eggs (Jegede *et al*., 2009, Kollins *et al*., 2012, Olayemi *et al*., 2019). The ovicidal and larvicidal effects observed on *H. contortus* could be attributed to the phyto-chemicals such as tannins present in the pods and tannins contained in plants possess anthelmintic activities (Ademola *et al*., 2004, Paolini *et al*., 2003, Paolini *et al*., 2005).

Tannins are also responsible for larval starvation of parasites because they have the ability to bind free protein available for larval nutrition and when this occurs it results into depletion of nutrients of the feeding larvae in the host, larval starvation and mortality of the parasites (Athanasidou *et al*., 2001). Previous studies revealed that plant extracts contains chemical compounds, anthelminthic activities and have inhibitory effects on the viability and hatchability of parasitic gastrointestinal eggs (Hounzangbe-Adote *et al*., 2005).

In conclusion, *in vitro* studies only measure the direct ovicidal and larvicidal inhibitory effects of the anthelmintic activities of these plants phyto-chemical compounds on parasites without interfering with the metabolic activities and physiological functions of the host (Githiori, 2004).
However, more detailed *in vivo* studies will help to establish the mechanism and degradation pathways, therapeutic effects, safety and toxicity profile of this plant extracts on the host.

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CONFLICT OF INTEREST

The authors do not have any conflict of interests to declare.

REFERENCES

Ademola IO, Fagbemi SO, Idowu SO. Evaluation of anthelmintic activity of *Khaya senegalensis* extract against gastrointestinal nematodes of sheep: *in vitro* and *vivo* studies.*Vet Parasitol* 122: 151–164, 2004.

Ademola IO, Fagbemi BO, Idowu SO. Anthelmintic activity of Extracts of *Spondias mombin* against gastrointestinal nematodes of sheep: Studies *in vitro* and *vivo.* Trop *Anim Health Prod* 37: 223– 235, 2005.

Aderogba MA, Okoh EK, Okeke IN, Olajide AO, Ogundaini AO. Antimicrobial and anti-inflammatory effects of *Piliostigma reticulatum* leaf extract. *Int J Pharmacol* 2: 70-74, 2006.

Akhtar M, Igbal Z, Kahn MN, Latef M. Anthelmintic activity of medicinal plants with particular reference their use in animal in the Indo-Pakistan subcontinent, *Smal Rmt Res* 3: 99-107, 2000.

Akinpelu DA, Obuotor EM. Antibacterial activity of *Piliostigma thonningii* stem bark. *Fitoterapia* 71: 442–443, 2000.

Alawa, C, Adamu A, Gefu J, Ajanusi, O, Abdu P, Chiezey N. *In vivo* efficacy of *Vernonia amygdalina* (compositae) against natural helminth infection in Bunaji (*Bos indicus*) calves. *Pakistan Vet J* 30: 215-218, 2010.

Asuzu IU, Onu OU. Anthelmintic activity of the ethanolic extract of *Piliostigma thonningii* bark in *Ascaridia galli* infected chickens. *Fitoterapia* 65: 291–297, 1994.

Asuzu IU, Gray AI, Waterman PG. The Anthelmintic activity of D-3-0- Methylchiroinositol isolated from *Piliostigma thionningii* stem bark. *Fitoterapia* 70:779, 1999.

Athanasiadou S, Kyriazakis I, Jackson F, Coop RL. Direct Anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. *Vet Parasitol* 99: 205–219, 2001.

Badar N, Iqbal Z, Khan M, Akhtar M. *In vitro* and *in vivo* anthelmintic activity of *Acacia nilotica* (L.) willd. ex delile bark and leaves. *Pakistan Vet J* 31: 185-191, 2011.

Biffa D, Narfeta A, Jobre Y. Evaluation of anthelmintic activities of crude leaf extract of the three indigenous herbal plants against ovine gastrointestinal nematodosis. *Ethopian Vet J* 8: 57-66, 2004.

Coles GC, Bauer C, Borgsteede FH, Geerts S, Klei TR., Taylor MA, Waller PJ. World Association for Advancement of Veterinary Parasitolog (WAAVP) methods for detection of anthelmintic resistance in nematodes of veterinary importance. *Vet Parasitol* 44: 35-44, 1992.

Debella A. Manual for phytochemical screening of medicinal plants, Department of Drug Research. Ethiopia: *Ethopina health Nut Res Inst*, 2002.

Eguale T, Tilahun G, Gidey M, Mekonnen Y. *In vitro* anthelmintic activities of four Ethiopian medicinal plants against *Haemonchus contortus*. *Pharmacol online* 3: 153-165, 2006.

Encyclopaedia Britannica.inc, (2018): http://www.britannica.com

Enejoh OS, Suleiman MM, Ajanusi JO, Ambali FS . Anthelmintic activity of extracts of *Citrus aurantifolia* (christm) fruit peels against experimental *Heligmosomoides bakeri* in mice. *J Adv Sci Res 6* : 29-32, 2015.

Fentahun T, Luke G. Small ruminant haemonchosis: Prevalence and associated determinants in randomly selected restaurants and hotels of Gondar Town Ethiopia. *Europ J Appl Sci* 4: 68-172, 2012.

Ferreira LE, Castro PMN, Chagas ACS, Franca SC, Beleboni RO. *In vitro* anthelmintic activities of Aqueous leaf extract of *Annona muricata L*. (Annonaceae) against *Haemonchus contortus* from sheep. *Exp parasitol* 134: 327-332, 2013.

Githiori JB. Evaluation of anthelmintic properties of Ethno veterinary plants preparation used as livestock dewormers by pastoralist and small holders farmers in Kenya, Doctoral thesis Swedish University of Agriculture Science Uppsala, Sweden. 2004.

Hassan MM, Hoque MA, Islam SKMA, Khan, SA Hossain, MB, Banu, Q. Efficacy of anthelmintics against parasitic infections and their treatment effect on the production and blood indices in Black Bengal goats in Bangladesh. *Turk J Vet Anim Sci* 36: 400- 408, 2012.

Hounzangbe-Adote FI, Mountairou K, Hoste H. *In vitro* effects four tropical plants on three life cycle stages of the parasitic nematode, *Haemonchus contortus*. *Res Vet Sci* 78: 155–160, 2005.

Ijaz M, Khan MS, Avais M, Ashrafi K, Ali MM, Saima. Infection rate and chemotherapy of various helminths in diarrheic sheep in and around Lahore. *J Anim Pl. Sci* 19: 13-16, 2009.

Jegede OC, Agbede RIS, Ajanusi JO, Adaudi AO. Anthelmintic screening of fractions of *Spigelia anthelmia* Linn extracts against experimental *Nippostrongylus braziliensis* in rats. *Vet arhive* 79: 189-197, 2009.

Jegede OC, Rabiu BM, Obeta SS, Malang SK, Ejiofor CE. Gastrointestinal parasites of ruminants slaughtered in Gwagwalada Abattoir, Federal Capital Territory, Abuja, Nigeria. *Nig J Parsitol* 34: 55-59, 2013.

Jimoh FO, Oladiji AT. Preliminary studies on *Piliostigma thonningii* seeds: proximate analysis, mineral composition and phytochemical screening. *Afr J Biot* 4: 1439–1442, 2005.

Kipyegon C. Anthelmintic activity of microencapsulated *Prosopis juliflora*(sw.) extracts against *Haemonchus contortus* of sheep. A Thesis Submitted in Partial Fulfillment for the Degree of Master of Science in Zoology (Animal Parasitology) of Jomo Kenyatta University of Agriculture and Technology, 2017.

Kollins EN, Wabo PJ, Payne VK, Jeannette Y, Marie-Claire K. *In vitro* Comparative Effect of Aqueous (Cold and Hot Water) and Ethanolic Extracts of the Seeds of *Aframomum Danielli* (Zingiberaceae) on Three Life Cycle Stages of the Parasitic Nematode *Heligmosomoides bakeri* (Nematoda; Heligmosomatidae), Parasite of the Laboratory Mice (*Mus Musculus*). *Med Arm. Pl* 1: 111, 2012.

Lemessa D. Piliostigma thonningii (Schumach.) Milne-Redh. Plant Resources of Tropical Africa, Wageningen, Netherlands. 2010, http://www.prota4u.org/search.asp

Maphosa V, Masika PJ, Bizimenyera ES, Eloff JN. *In vitro* anthelminthic activity of crude aqueous extracts of *Aloe ferox*, *Leonotis leonurus* and *Elephantorrhiza elephantina* against *Haemonchus contortus*. *Trop Anim Health Prod* 42: 301–307, 2010.

Martin RJ. Modes of action of anthelmintic drugs. *Vet J* 154: 11-34, 1997.

Nabi H, Saeed K, Shah SR, Rashid MI, Akbar H, Shezhad W. Epidemiological study of gastrointestinal nematodes of goats in District Swat, Khyber Pakhtunkhwa. *Pakistan Sci Int* 26:2 83- 286, 2014.

Olayemi DO, Onakpa MM, Jegede OC. Anthelmintic Activity of *Hymenodictyon Pachyanta* Stem bark extracts against *Haemonchus contortus*. *FOLIA VETERINARIA* 63:30-36, 2019.

Paolini V, Dorchies PH, Host H. Effects of sainfoin hay on gastrointestinal infection with nematodes in goats. *Vet Recd* 152: 600–601, 2003.

Paolini V, De La Farge F, Prevot F, Dochies PH, Hoste T. Effects of the repeated distribution of sainfoin hay on the resistance and the resilience of goats naturally infected with gastrointestinal nematodes. *Vet Parasitol* 127: 277–283, 2005.

Schoenian S, Integrated parasite management. Maryland Cooperative extension. Maryland Small Ruminant 2003, www.sheepandgoat. com/articles/IPM.html.

Silva O, Barbosa S, Diniz A, Valdeira ML, Gomes E. Plant extracts antiviral activity against Herpes simplex virus type 1 and African swine fever virus. *Int J Pharmacog* 35: 12–16, 1997.

Simon MK, Ajanusi OJ, George BD, Abubakar MS, Meduna JA. *In vivo* evaluation of the stem bark of *Combretum molle* (R. Br. x. G. Don) for anthelmintic properties. *Cont J Vet Sci* 2: 1–1, 2008.

Simon MK, Jegede OC, Nafarnda WD. Seasonal influence on *Haemonchus contortus* infection and response to treatment with albendazole in Yankasa lambs. *Adv Appl Sci Res* 3: 2243-2246, 2012.

Squires JM, Ferreira JFS, Lindsay DS, Zajac AM. Effects of Artemisinin and Artemisia extract on *Haemonchus contortus* in gerbils *(Meriones unguiculatus). Vet Parasitol* 10: 103–108, 2011.

Swarnakar G, Bhardawaj B, Sanger B, R. Prevalence of gastrointestinal parasite in Cow and Buffalo of Udaipur district. India. *Int J Cur Micro Appl Sci* 4: 897-902, 2015.
\
Taylor MA, Coop RL, Wall RL. Vet Parasitol, 2007, 3rd Ed.Blackwell Publishing Ltd, Oxford, UK, pp. 872.

Travelmath. The latitude and longitude of Zaria, Nigeria. http://www.travelmath.com/city/zaria, Nigeria, 2011.

Wabo PJ, Yondo J, Fossi TO, Marie CK, Bilong BCF, Mpoame M. The *in vitro* effects of *Chenopodium ambrosioides* (Chenopodiaceae) extracts on the parasitic nematode *Heligmosomoides bakeri* (*Nematoda, Heligmosomatidae*). *J Pharmacog Phyto* 3: 56-62, 2011.

Supplementary Table 4: Mean (±SD) percentage inhibition of egg hatch of *H. contortus* at different concentration of *P. thoningii* fraction (mg/ml) and Albendazole (mg/ml).

Fractions	Treatments	0.78mg/ml	1.56mg/ml	3.125mg/ml	6.25mg/ml	12.50mg/ml
N-Butanol	*P. thoningii*	90.5±2.29[a]	91.67±2.08[a]	92.17±0.76[a]	95.5±0.86[a]	97.67±0.763[a]
Ethyl acetate	*P. thoningii*	93.5±2.78[c]	94.67±2.57[c]	96.83±2.31[b]	98.17±1.76[b]	100±0.00[b]
Petroleum Ether	*P. thoningii*	91.33±2.31[d]	93.1±1.85[d]	95.17±1.53[c]	97.27±2.19[b]	99.73±0.46[b]
Aqueous	*P. thoningii*	88±1.0[e]	90.83±1.04[e]	92±1.32[d]	93.23±1.5[c]	99.17±0.76[b]
Albendazole		96.83±1.04[b]	98 ± 0.50[b]	99.0 ± 0.00[b]	99.83 ±0.28[b]	100 ± 0.00[b]
DW	6.2 ± 2.33					

Means with different superscript letter (a, b, c, d, e) differ significantly (p<0.05) from the positive control group
DW: Distilled water

Supplementary Table 5: Mean (±SD) percentage inhibition of larval development of *H. contortus* at different concentration of *P. thoningii* fraction (mg/ml) and Albendazole (mg/ml).

Fractions	Treatments	0.78mg/ml	1.56mg/ml	3.125mg/ml	6.25m/ml	12.50mg/ml
N-Butanol	*P. thoningii*	67.0±3.0[a]	73.0±2.0[a]	78.0±2.0[a]	84.0±2.65[a]	96.0±4.36[a]
Ethyl acetate	*P. thoningii*	68.5±2.0[c]	74.0±2.0[c]	80.0±4.0[c]	85.0±5.0[c]	96.93±0.75[c]
Petroleum Ether	*P. thoningii*	58.0±4.0[d]	62.2±2.0[d]	70.0±2.0[d]	79.2±2.0[d]	89.0±2.0[d]
Aqueous	*P. thoningii*	72.5±3.0[b]	77.0±2.0[b]	85±3.0[e]	89.0±0.0[e]	98.2±1.8[e]
Albendazole		73.47±2.25[b]	77 ± 1.73[b]	91.3 ± 3.2[b]	100.0 ±0.0[b]	100 ± 0.00[b]
DW		12. 3 ± 1.45				

Means with different superscript letter (a, b, c, d, e) differ significantly (p<0.05) from the positive control group
DW: Distilled water