# *IN VITRO* ANTHELMINTIC ACTIVITY OF GOAT BILE CHEMICAL SUBSTANCES AGAINST *Haemonchus contortus*

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Abstract: Extracts of goat bile Chemicals substances were investigated for anthelmintic activity against *Haemonchus concortus*. Various concentrations (50, 100 and 200 mg/ml) of each extract of the bile were tested using the egg hatch assay and larval development assay. All extract tested showed 100% mortality at the end of 180 min of the test duration. While 0.25 mg/ml of albendazole the positive control showed 100% mortality after 120 min. The results of the egg hatch inhibition assay showed that at a concentration of 200 mg/ml, 65% of the eggs were inhibited from hatching by the bile extract. Albendazole inhibited 98% of the eggs from hatching at 0.25 mg/ml. Goat bile extract has potential as anthelmintic from nature, that can be explore in the search for natural remedy for parasitic affections.

**Keywords:** Albendazole, anthelmintic activity, egg hatching, goat bile, *Haemonchus concortus*

## Introduction

Helminthiasis which is caused by the helminthes infection is proved to be a major constrain in the livestock production all around the globe. The parasitic disease affects the health status of a large fraction of human population as well as animals (Maity *et al*., 1998). In developing countries, they pose a large threat to public health and contribute to the prevalence of malnutrition, anemia, eosinophilia and pneumonia (Bundy, 2004). Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world (Cole, 1997: Geert & Dorny, 1995). However, development of resistance in helminthes against conventional anthelmintics is a foremost problem in the treatment of helminthes diseases (Tagbota *et al*., 1994). Henceforth it is important to look for alternative strategies against gastrointestinal nematodes, which have led to the proposal of screening medicinal plants for their anthelmintic activity (Yoganandam *et al*., 2010).

Literature surveyed reveals that goat bile can be used to treat various kinds of gastrointestinal problems. Goat bile primarily consists of water, salts, cholesterol and a fatty substance (Barrett, 2012; Guyton and Hall, 2011). One of its main roles is to help the animal absorb vitamins from food that has been ingested, Goat biles were believed to be effective in treating optic atrophy, including acute hemorrhagic conjunctivitis, marginal suppurative blepharitis, and epiphora (Tao, 1955). Goat bile was also used to treat temporary blindness following a life-threatening illness (which was most likely smallpox), and eye injury from foreign bodies (ref). These biles were believed to be effective in ameliorating various infectious skin diseases, chancre in children (most likely impetigo), and constipation (ref). When compounded with pig pancreas and asarum herb, these biles were also prescribed as a facial lotion for minimizing chloasma in pregnant women and to derma base freckles (Li, 1957).

Also, when goat bile was decocted thrice with ox bile and wines the resulting liquor was believed to be effective in reversing any olive discoloration of the skin from itchy dermatitis, which was most likely secondary to tinea versicolor (Jiangsu, 1977). Therefore, the aim of this work is to evaluate the anthelminthic potentials of goat bile on *Heamonchus contortus*.

## Materials and Methods

### *Sample collection*

The one litre of goat bile was obtained from abattoir in Randan Kano, Zaira, Kaduna state of Nigeria in the month of in July 2018. The goat bile sample was preserved with Methanol (40%) until needed for extraction.

### *Heamonchus contortus lavar*

Adult *H. contortus* were recovered from the abomasums of freshly slaughtered goat in a local abattoir in Randan Kano, Zaria in the month of July, 2018. Which were confirmed by a Parasitologist at the Department of Parasitology, Faculty of Veterinary, Ahmadu Bello University, Zaria, Nigeria. All female *H. contortus* were morphologically selected and macerated to liberate the eggs. Distilled water was added and the eggs were successively sieved using Baermann funnel.

### *Extraction of Goat Bile*

The bile (1 Litre) containing methanol (40%) was acidified with concentrated tetraoxosulphate (VI) acid ($H_2SO_4$), until the bile mixture was acidic to litmus paper. The observed precipitate (**BP-A**) was filtered. The filtrate was extracted with chloroform and concentrated under vacuum using a *rota vapor* at 40°C, to give chloroform bile extract **(CBE-1**). The aqueous layer was basified with NaOH until it was basic to litmus paper, the observed precipitate **(BP-B)** was filtered and the resulting aqueous layer extracted with chloroform and concentrated as earlier mention to give a second fraction (**CBE-2**). The precipitate BP-A and BP-B were suspended in ethylacetate and concentrated to remove any traces of water.

### *Biological studies*

### *Larval motility test (LMT)*

Lava mortality test was conducted following the technique describe by Fernandez *et al.* (2009). Briefly; a total of about 368 lava parasites were used in the study. Three concentrations were employed for each extract. Ten worms were exposed in triplicate to each of the following treatments in separate Petri dishes at room temperature (25 – 30°C). There were 5 groups as follows; CBE-1, CBE-2, BP-A, albendazole (positive control) and water (negative control). The motility of worms was observed and mobile worms were counted at different time intervals till 7 h post treatment. Worms not showing any motility were picked out and kept in lukewarm phosphate buffer saline (PBS) for 10 min and, in case of revival in motility, the observed worms were counted as alive; otherwise, they were counted as dead. The Mortality rate for each concentration of the compound was determined using the ratio;

$$\frac{Total\ No.of\ initial\ larvae\ in\ wells - total\ No.of\ dead\ larvae\ in\ well}{Total\ number\ of\ initial\ larvae\ in\ wells}\ x\ 100$$

***Egg hatch inhibition assay (EHIA)***

Freshly collected adult female *H. contortus* were picked, crushed and sieved to obtain the eggs, which were then triturated in PBS. The suspensions were centrifuged for 2 min at 300 rpm and the sediment was retained. This sediment was resuspended in saturated solution of NaCl to form a convex meniscus above the test tube. After putting a coverslip above the test tube, the samples were centrifuged again. Coverslip was carefully removed and eggs were washed into another test tube. This solution was then centrifuged and eggs were collected from sediment. Eggs were washed thrice with distilled water and adjusted to a concentration of 100 – 200 eggs/mL, using the McMaster technique (Soul, 1982). Egg Hatch Inhibition Assay was performed following the technique of Coles *et al*. (1992). Approximately, 100 eggs in 200 $\mu$l of water were pipetted into each well of a 48-well microtiter plate. To each of the test wells, 200 $\mu$l of each bile extract at different concentrations (50, 100 and 200 mg/ml) was added to a final volume of 400 $\mu$l per well. Similarly, 200 $\mu$l of albendazole (standard drug) at 0.25 mg/ml concentration and distilled water were used as positive and negative controls, respectively. Each test was carried out in three replicates and the results were expressed as mean ± SD. The plates were incubated in a humidified incubator at 37°C for 48 h. Thereafter, a drop of Lugol's solution was added to stop further hatching. All unhatched eggs and larvae (L1) in each well were counted. The percent inhibition of egg hatching was calculated using the formula below (Coles et al., 1992). The percentage of hatched eggs was calculated using the ratio;

$$\frac{Total\ initial\ No.\ of\ eggs\ in\ wells - hatched\ eggs\ in\ well}{Total\ number\ of\ initial\ eggs\ in\ wells} \ x\ 100$$

**Results and Discussion**

This study indicated that the bile extracts produced a relatively comparable anthelmintic activity with the conventional anthelmintic, albendazole. The activity increased with concentration and time. After 3 hours of exposure of Lavar of *H. contortus* to different concentrations of the bile extracts a dose-dependent increase in motility was observed for bile extracts (Table 1). At highest concentration (200 mg/mL), the bile extracts produced mortality of the larva *H. contorts* ranging 9 and 100% after 3 h exposure to the extracts (Table 1). Albendazole, on the other hand killed the parasites in a time-dependent manner and all the lavar worms were dead at a concentration of 0.25 mg/mL within 3 h of exposure.

**Table 1: Percentage mortality of *Haemonchus contortus***

| Fractions | Concentration (mg/ml) | Mortality (Percentage ± SD) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 30 min | 60 min | 90 min | 120 min | 150 min | 180 min |
| CBE-1 | 200.0 | 12.7±3.35 | 63.7±2.65 | 87.7±5.65 | 100 | 100 | 100 |
| | 100.0 | 9.4±3.30 | 17.33±3.34 | 36.7±4.65 | 100 | 100 | 100 |
| | 50.0.0 | 0.00 | 11.7±6.65 | 13.0 ±3.0 | 46.0±4.00 | 85.4±5.30 | 100 |
| CBE-2 | 200.0 | 18.0±2.00 | 40.4±1.70 | 88.4±2.30 | 100 | 100 | 100 |
| | 100.0 | 14.7±5.85 | 35.0±8.65 | 59.7±1.63 | 88.7±2.00 | 100 | 100 |
| | 50.0 | 11.0±1.00 | 16.4±7.00 | 36.7±4.65 | 55.7±4.35 | 65.7±5.25 | 100.0 |
| BP-A | 200.0 | 23.0±4.70 | 50.0±2.00 | 82.0±2.00 | 92.0±2.00 | 100 | 100.0 |
| | 100.0 | 17.4±2.70 | 44.0±2.00 | 61.33±5.60 | 87.7±2.60 | 90.0±2.00 | 100.0 |
| | 50.0 | 10.0±4.00 | 24.4±15.1 | 34.7±3.35 | 50.0±2.00 | 80.0±2.00 | 100.0 |
| Albendazole | 0.25 | 50 | 80 | 90.0 | 100.0 | 100.0 | 100.0 |
| Water | 1 ml | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

0.0 = No mortality

The result of egg hatch inhibition assay at graded concentration of bile extracts of is shown in Table 2. The result indicated that all the extracts produced a relatively comparable egg hatching inhibitory effect with albendazole. At a maximum concentration of 200 mg/ml CBE-1, CBE-2 and BPE-A induce egg hatch inhibition of 47, 59 and 64% while albendazole induced 98% egg hatching inhibitory at 0.25 mg/ml. Table 2.

**Table 2: Result of effect of the test samples at varying concentrations on egg hatching assay of H. *contortus***

| Samples | Conc. (mg/ml) | Percentage inhibition |
|---|---|---|
| **CBE-1** | 50 | 0.00 |
| | 100 | 26.0±1.80 |
| | 200 | 47.0±10.35 |
| **CBE-2** | 50 | 0.00 |
| | 100 | 22.2±1.65 |
| | 200 | 59.6±6.5 |
| **BPE-A** | 50 | 0.00 |
| | 100 | 47.7±1.70 |
| | 200 | 64.5±7.30 |
| **Albendazole** | 0.25 | 98±2.30 |
| **Water** | 0.00 | 0.00 |

*In vitro* tests to evaluate the inhibition of egg hatching and larval motility are widely used in veterinary parasitology to the prospecting of novel anthelmintic agents (Costa *et al*., 2002; Vasconcelos *et al*., 2007). The advantage of these assays is that compounds or materials to be tested are in direct contact with the different life-cycle stages of the parasite. Effective anthelmintic agents should inhibit worm egg hatching and larval motility by more than 90% and when inhibiting 80 – 90% should be considered moderately effective. Thus, our in vitro results obtained with the goat bile extract against *H. contortus* eggs and larvae, particularly in the higher dilutions, allow us to classify the tested extract as moderately effective (Ferreira *et al*., 2013). The bile extract showed a statistically significant anthelmintic activity against the eggs and larvae life-cycle stages of the parasite. This finding is significant since, theoretically, it reduces the chances of the occurrence of resistance of the parasite when using the extract in clinical practices due to anthelmintic action on different phases of worm development (Hounzangbe-Adote *et al*., 2005). Studies have suggested that the mechanism of inhibition of egg hatching and larval development of different parasites is related to the inhibition of cell division and, consequently, to the formation and development of vital structures of the parasite (Gallardo *et al*., 1998). Treatment of nematode infections using conventional

anthelmintic drugs resulted in about 294 million dollars of veterinary market revenue in 2004 (Molento *et al*., 2004). The consequences of this situation go beyond the rising costs of livestock management. In addition to the problem of resistance discussed above, there is no clear evidence that synthetic anthelmintics leave no residues in meat that would pose potential public health hazards (Rodrigues *et al*., 2007). Therefore, the identification of novel promising anthelmintic compound from bile extracts may contribute for the development of therapeutic products that could be more cost effective, safer, and more accessible and provide a lower risk of resistance than the conventional therapeutic arsenal currently employed. The *in vitro* tests using free living stages of parasitic nematodes offer a means of evaluating the anthelmintic activity of new compounds (Asase *et al*., 2005). *In vitro* techniques are preferred to *in vivo* methods due to their low cost, simplicity, and rapid turnover (Markus and Ernst, 2005). The bile chemical substance is documented to possess medicinal properties in ethnobotanical surveys conducted by ethnobotanists in traditional system of medicine (Wang and Carey, 2014). The present study showed 100% efficacy of the bile chemical substance extract against the parasite at the concentration of 200 mg/ml which was the highest efficacy value was comparable to the standard anthelmintic, albendazole. The egg hatching inhibitory effect of bile chemical substance extract was 64.5% at the concentration of 200 mg/ml. This is evident from the current study, which showed 95% of mortality of lavar parasites of *H. contortus* at a concentration of 200 mg/ml in all extracts of the goat bile chemical substance. The egg hatching inhibitory effect was highest (64%) at a concentration of 200 mg/ml for the BPE-A extracts of the goat bile chemical substance. Increment on the concentration of the extracts of the goat bile chemical substance resulted in increased inhibition of egg hatching indicating dose-dependent activity. Chemical substances like alkaloid and tannin have been reported to responsible for anthelmintic activity. The observed anthelmintic activity of plant extracts in the present study may be due to these chemical substances being present in the bile chemical substance (Debella, 2002; Da Silva, 2008).

Finally, the *in vitro* methods provide a means to screen rapidly for potential anthelmintic activities of different plant extracts. Due to drug biotransformation, interaction with food materials, and absorption variations, the results obtained by the *in vitro* method could not be extrapolated to *in vivo* activity. Therefore, results should be ascertained by *in vivo* evaluation. In conclusion, the current study showed that extracts of the bile chemical substance a promising *in vitro* anthelmintic activity against lavar and oval stages of *H. contortus.* Fractionation of the each extracts o of the bile chemical substance and further anthelmintic efficacy studies involving other parasite development stages are warranted.

**Conflict of Interest**

Authors declare that there is no conflict of interest reported in this work.

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