



In vitro anthelmintic activities of three ethnomedicinal plant extracts against *Haemonchus contortus*

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Abstract

Aim of study: To investigate the *in vitro* anthelmintic efficacies of three plants, *Annona senegalensis* (AS), *Cochlospermum planchonii* (CP), and *Sarcocephalus latifolius* (SL), used by livestock farmers in Northern Nigeria, to treat gastrointestinal nematode infections in ruminants.

Area of study: Makurdi, Benue State, Nigeria.

Materials and methods: The plants were selected through a structured questionnaire administered to livestock farmers in Northern Nigeria. Aqueous and acetone leaf (AS and SL) and root (CP) extracts of these plants were investigated for their anthelmintic activity against *Haemonchus contortus* using the egg hatch inhibition assay at concentration levels of 0.3125 to 10 mg/mL in 2.5% dimethyl sulfoxide.

Main results: A probit log-dose response analysis showed that acetone extract of CP achieved 100% egg hatch inhibition similar to the commercial drug at all tested concentrations after 48 hours of incubation, while AS demonstrated 88.7% egg hatch inhibition. Acetone extract of SL had less than 50% egg hatch inhibition at all tested concentrations. On the other hand, the aqueous extract of CP and SL both exhibited 100% inhibition at 5 and 10 mg/mL of the tested concentrations, while AS had less than 50% egg hatch inhibition at all tested concentrations.

Research highlights: This study identified CP, AS, and SL as medicinal plants with rich sources of molecules that have potential value in the development of novel anthelmintic drugs.

Additional keywords: natural products; lead anthelmintics; botanicals; *Cochlospermum planchonii*; *Annona senegalensis*; *Sarcocephalus latifolius*; egg hatch; gastrointestinal nematodes.

Abbreviations used: DMSO (dimethyl sulfoxide); EHA (egg hatch assay).

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Introduction

Parasitic gastrointestinal nematode infections remain a leading factor militating against the productivity and profitability of livestock business, especially the small ruminant

enterprise, all over the world. In the tropics, these infections are mainly caused by the trichostrongylid nematodes, particularly, *Haemonchus contortus*, a blood-sucking nematode (Saddiqi *et al.*, 2011). Infections are often associated with significant economic losses ranging from insidious loss of

body conditions to outright mortality. The infection by this parasite is often widespread and occurs all year round in the tropics (Chiejina, 1986; Sowemimo *et al.*, 2012). High *H. contortus* burden may lead to death especially in young animals. Under field conditions, co-infection with other species of the nematode sometimes occurs (Squire *et al.*, 2019).

The control methods available to farmers in the tropics involve reducing worm burden in the animal through anthelmintic drug treatment in combination with controlled grazing, which consequently reduces the contamination of pastures (Barger, 1999). However, communal ownership of farmlands in most communities in the tropics limits their use for controlled grazing (Githiori *et al.*, 2003). Consequently, anthelmintic intervention remains the most effective means of controlling the disease, but their continuous use and efficacy are limited by the unaffordability of the drugs, their uncertain availability, and the emergence of worm strains that are resistant to the available drugs (Epe & Kaminsky, 2013). In addition, there has been an increasing concern over chemical residues in edible animal products associated with the use of anthelmintic drugs in livestock (Waller, 1997).

Anthelmintic control of helminths involves routine treatment with synthetic drugs belonging to different anthelmintic families, namely, benzimidazoles, imidazothiazoles, and macrocyclic lactones. This has inevitably led to the selection of resistant strains of gastrointestinal nematode parasites, and particularly *H. contortus*, which resulted in partial or total inefficacy of most anthelmintic classes (Roeder *et al.*, 2013). Given the lack of prospect of developing a vaccine against these parasites and the economic impacts caused by the increasing resistance to anthelmintics, it is important to either discover novel molecules or compounds able to control multi-resistant nematodes or seek viable alternatives that are effective, affordable, safe, and less selective for resistant worms.

Medicinal plants have served as a constant source of remedies for a variety of diseases over centuries. Plants have been a rich source of antimicrobial and anthelmintic agents and their products are used medicinally in different parts of the world as sources of many potent and safe drugs, including anthelmintics (Lai *et al.*, 2005; Abdul-Ghani *et al.*, 2011; Harvey *et al.*, 2015; Irum *et al.*, 2015). This study was therefore designed to investigate the anthelmintic efficacies of three medicinal plants, namely, *Annona senegalensis*, *Cochlospermum planchonii*, and *Sarcocephalus latifolius* against *H. contortus*.

Material and methods

Plant collection

The plants used in this study were selected through a structured questionnaire administered to livestock farmers

in Northern Nigeria. The questionnaire elicited information on herbs used in the treatment of animal diseases, of which the three plants were among those used by farmers to treat gastroenteritis, including those due to nematode parasitism in ruminants. The plants were collected within the vicinities of the University of Agriculture, Makurdi, Benue State. They were identified by a plant taxonomist in the Department of Botany, University of Agriculture, Makurdi, where voucher specimens were deposited in an herbarium. The plant materials, which include the root of CP and leaves of AS and SL were air-dried at room temperature (22-37°C) and relative humidity of 39-45.6% for 6-8 days. Thereafter, the dried samples were ground to a fine powder with a hammer mill and then stored in an air-tight container at room temperature until needed.

Plant extraction

Acetone and aqueous (water) extraction were performed on each of the three plant materials. Acetone was used because of its ability to extract compounds with a wide range of polarities based on its superiority as an extractant, and on several parameters, as described in several studies (Eloff, 1998; Kotze & Eloff, 2002; Eloff *et al.*, 2005). One gram of each of the plant materials was separately extracted with 10 mL of acetone (>99% technical grade, Merck) in polyester centrifuge tubes. Aqueous extraction of the plants was chosen, because water is the solvent used by the natives. The tubes were vigorously shaken on an orbital shaker for 30 minutes, and then centrifuged at 4000 × g for 10 minutes. Thereafter, the supernatant of each extraction was filtered using Whatman No.1 filter paper into pre-weighed glass containers. The solvents were allowed to evaporate under a stream of air in a fume hood at room temperature to obtain the dried extract. The extracts were stored at 4 °C until required and reconstituted in 5% dimethyl sulfoxide (DMSO) when needed for the assay.

Recovery and preparation of *H. contortus* eggs for egg hatch assay

H. contortus eggs used in the assay were obtained from faeces collected per rectum from goats carrying mono-specific infections of *H. contortus*. Approximately 3 g of the faecal sample were crushed and made relatively liquid (slurry) by adding 42 mL of saturated sodium chloride solution, and the suspension passed through a tea strainer. The filtrate was placed in 15 mL test tubes on the bench undisturbed for 15 minutes to allow the worm eggs to float to the top of the tube. Thereafter, the top portions of the fluid containing the eggs were poured into another tube which was then resuspended with water to dilute and wash out the salt solution. The tubes were centrifuged at ×1000 g for 5 minutes after which the supernatant was decanted and the

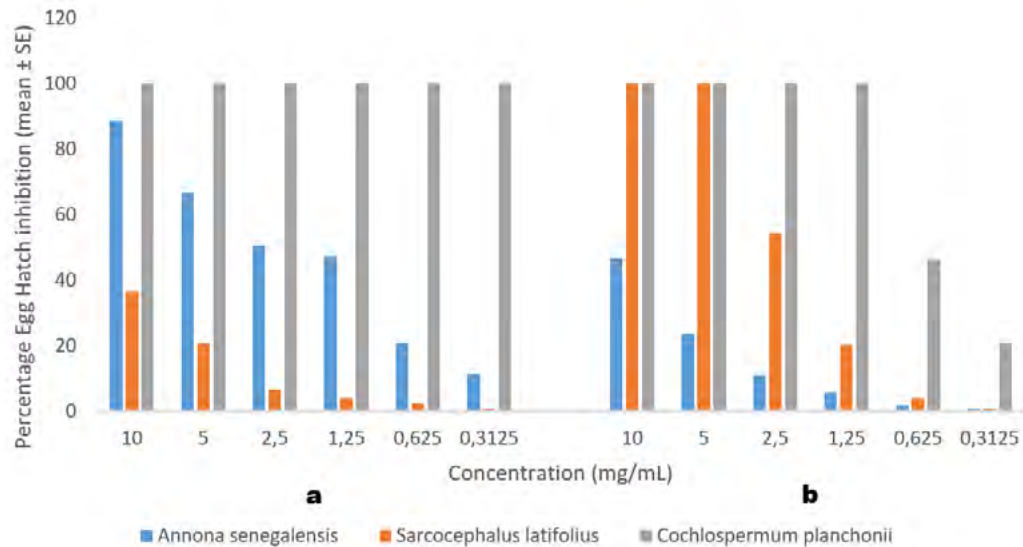


Figure 1. Mean percentage egg hatch inhibition of different concentrations of acetone (a) and aqueous (b) *C. planchonii* root, and leaves of *A. senegalensis* and *S. latifolius* extracts. Albendazole was a positive control and recorded 100% inhibition at all concentrations (0.01 to 25 μ g/mL) used.

sediment resuspended with water. This was repeated three times after which the ‘cleaned’ eggs were resuspended with deionized water and placed in a universal bottle. For use in the assay, 1 mL of the egg suspension was further diluted to contain approximately 100 eggs in 200 μ L. Egg count was carried out using the modified McMaster egg counting technique as described in Fakae *et al.* (1999).

Egg Hatch Assay (EHA)

Egg hatch assay (EHA) was conducted following the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles *et al.*, 1992). Approximately 100 *H. contortus* egg suspension in 200 μ L of deionized water was incubated with different concentrations (0.625, 1.25, 2.5, 5.0, 10.0 and 20 mg/mL) of each plant extract in 5% DMSO in a 48 –flat-bottomed microtitre plate to obtain a final tested concentration of 0.3125 to 10 mg/mL in 2.5% DMSO. Albendazole served as a positive control and was dissolved in 5% DMSO in de-ionized water to obtain different concentrations (0.01 to 25 μ g/mL), while 5% DMSO served as the negative control. The setup was incubated in triplicate for each extract at 27°C for 48 hours. At the end of 48 hours, a drop of Lugol’s iodine solution was added to each well and the number of larvae vs unhatched eggs (including larvated ones) was counted.

Thereafter, Probit analysis (Finney, 1971) was conducted to determine the lethal concentration (LC_{50}) of the extracts and albendazole. The percentage inhibition of egg hatching was calculated using the formula by Cala *et al.* (2012):

$$E = 100 [(Eggs + L1) - L1] / (Eggs + L1),$$

where E = % inhibition of egg hatching, and L1 = Number of larvae in a particular well. All experiments were undertaken in triplicate on three separate occasions.

Results

The *in vitro* anthelmintic activity of the acetone and water leaf extracts of AS, SL, and the crude water (aqueous) and acetone root extracts of CP were determined using the eggs of *H. contortus*. The results in Figs. 1 and 3 show that the extracts of all the plants had a dose-related percentage egg hatch inhibition, whereby activity increases as the concentration of the extract increases. The results have the acetone root extract of CP inhibiting egg hatch by 100% in all the concentrations used with an LC_{50} less than 0.3125 mg/mL (Fig. 1), which is the lowest concentration tested. This was followed by the acetone leaf extract of AS with a maximum inhibition of 88.7% at the highest concentration of 10 mg/mL. A probit log-dose response analysis showed that AS had an LC_{50} of 1.95 mg/mL (Fig. 2). Acetone leaf extract of SL did not produce up to 50% hatch inhibition in all the tested concentrations (Fig. 1).

The results of the egg hatch inhibition of the water extract of the plants are shown in Fig. 3. The results showed that water root extract of CP had the best inhibitory activity against the *H. contortus* eggs producing 100% inhibition in all the concentrations used (0.3125 to 10 mg/mL). The water extract of CP had 46.2 and 20.7% inhibitions at concentrations of 0.625 and 0.3125 mg/mL respectively. This was followed by the waterleaf extract of SL that

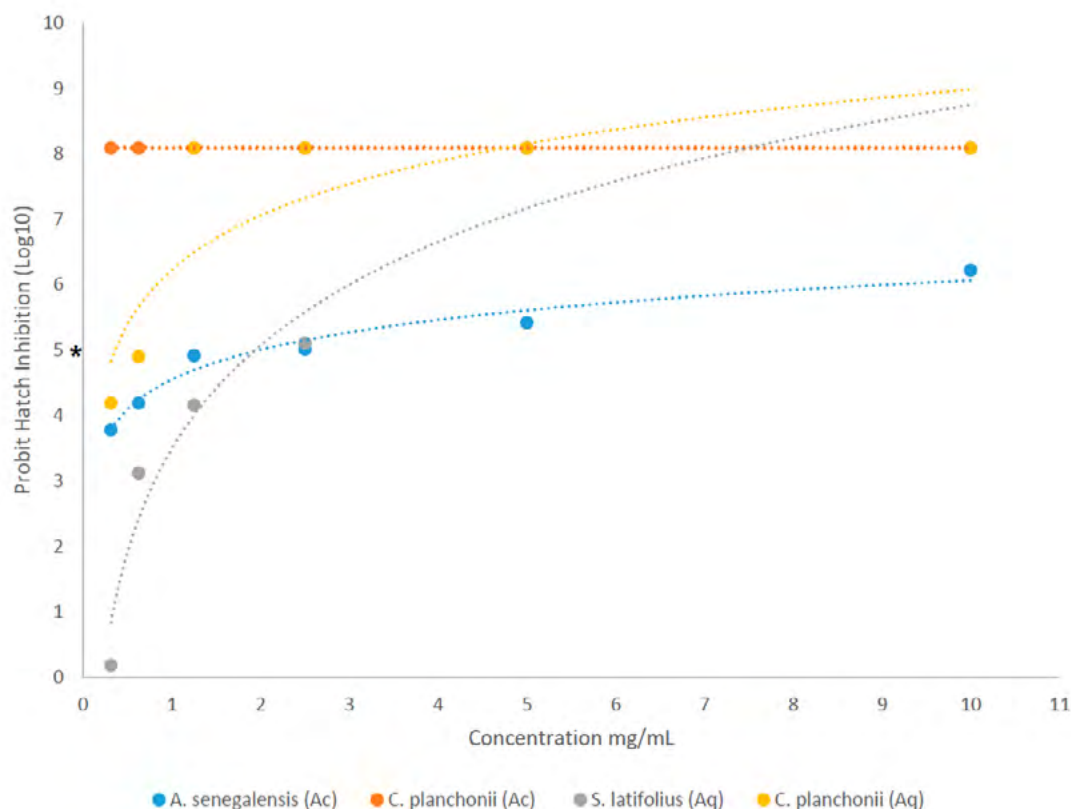


Figure 2. Probit hatch inhibition of *H. contortus* eggs after 48 hours incubation with different concentrations (0.3125-10 mg/L) of acetone (Ac) extracts of *C. planchonii* root, *A. senegalensis* leaves, and aqueous (Aq) extracts of *C. planchonii* root and *S. latifolius* leaves. * LC_{50} -Acetone extracts: *C. planchonii* <0.3125 mg/mL; *A. senegalensis* = 1.95 mg/mL. * LC_{50} -Aqueous extracts: *C. planchonii* <0.3125 mg/mL; *S. latifolius* = 2.5 mg/mL

inhibited 100% of the egg at concentrations ≥ 5 mg/mL. The waterleaf extract of AS had less than 50% egg hatch inhibitions at the tested concentrations (Fig. 3). A probit log-dose response analysis of the percentage hatch inhibition showed that water root extract of CP and the leaf extract of SL with EC_{50} of 0.63 and 2.5 mg/mL respectively (Fig. 4).

In all the assays, albendazole was used as the positive control and had 100% egg hatch inhibition at all concentrations (0.01 to 25 μ g/mL) used, while DMSO used as the diluent recorded <10% inhibition.

Discussion

This study investigated the anthelmintic efficacies of three plants used in folk medicine for the treatment of gastrointestinal worm infections, using the *in vitro* EHA. Efficacy was determined by the ability of extracts of CP, and SL and AS to inhibit the egg hatching of *H. contortus*. Among the plant extracts sampled, acetone root extracts of CP and AS leave, and water extracts of CP and SL had good anthelmintic activities. This suggests that the compounds responsible for the anthelmintic activity of CP and

AS may be lipid-soluble, while those of SL are more polar. In this study, plants with LC_{50} of ≤ 2.5 mg/mL representing one-quarter of the highest concentration used were considered to possess bioactive compounds against *H. contortus*. The study, therefore, identified acetone and water root extracts of CP as having high *in vitro* anthelmintic activity against *H. contortus*. Similarly, acetone and aqueous leaf extract of AS and SL respectively, were identified with good *in vitro* anthelmintic activity.

The acetone leaf extract of CP and albendazole which served as positive control showed a 100% egg hatch inhibition rate after 48 hours. The water root extract of CP equally inhibited 100% of the eggs from hatching at concentrations ≥ 1.25 mg/mL. This agrees with the report of Koné *et al.* (2005) which demonstrated that CP has an ovicidal activity against *H. contortus* eggs, in a study that investigated anthelmintic activity of medicinal plants used in northern Ivory Coast against intestinal helminthiasis. The result of the present study is considered very significant given that the extracts are crude samples with several compounds and can be a source of photochemical with anthelmintic activity compared to albendazole which is a standard anthelmintic. However, it remains to be seen

whether or not CP has the same level of activity against *H. contortus* and other gastrointestinal nematode parasites *in vivo*.

C. planchonii is a low shrub savanna plant that grows up to 2.0–2.5 m high and reproduces from seeds and rhizomes (Burkill, 1997). It is a common weed of cultivated fields in both Guinea and Sudan savanna zones. In Northern Nigeria, CP is used by the Fulani pastoralist to treat diarrhea and gastrointestinal nematode infections in animals, hence its inclusion in this study (Mhomga *et al.*, 2019). Phytochemical analysis of the acetone root extract of CP reveals the presence and percentage occurrence of tannins (13.5%), flavonoids (10%), alkaloids (7.3%), saponins (4.1%), and phenolic compounds (1.6%) (Mhomga *et al.*, 2018). The presence of these secondary metabolites, particularly, tannins, alkaloids, and saponins have been associated with anthelmintic activities in plants (Onyeyili *et al.*, 2001; Wang *et al.*, 2010; Cock, 2011).

A. senegalensis or African custard-apple is a potent medicinal plant generally used traditionally in the treatment of many diseases (Mustapha, 2013). Poultice of *A. senegalensis* Pers (Annonaceae) leaves is reported to be used for the treatment of worm infestation and diarrhea (Burkill, 1997; Fall *et al.*, 2008). The anthelmintic efficacy of AS recorded in the present study is consistent with previous studies that demonstrated the anthelmintic properties of AS. Alawa *et al.* (2003) investigated the efficacy of AS extract against *H. contortus* eggs and showed a significant concentration-dependent reduction in the egg hatch and larval recovery. Koné *et al.* (2005) reported an LC_{50} of 0.096 mg/mL with ethanolic root extract of AS against *H. contortus* in a study on the anthelmintic activity of medicinal plants used in northern Ivory Coast against intestinal helminthiasis.

In this study, the aqueous leaf extract of SL inhibited 100% of the eggs from hatching at 5 and 10 mg/mL concentrations with an LC_{50} of 2.5 mg/mL. This suggests that SL possess potent anthelmintic compounds, and consequently a potential rich source of lead molecule for development of new anthelmintic drug. Although the report of anthelmintic activity of SL in the literature is either scanty or nonexistent, studies have demonstrated saponins, flavonoids, alkaloids, and steroids as the important phytochemicals present in the plant leaves (Edewor *et al.*, 2015). These compounds, particularly saponins and alkaloids are assumed to be responsible for the anthelmintic activity observed in this study (Cock, 2011).

In conclusion, this study confirmed that *A. senegalensis*, *S. latifolius*, and *C. planchonii* have good anthelmintic properties and are rich sources of molecules that are of potential value in the development of novel anthelmintic drugs. *C. planchonii* had the best and most potent anthelmintic properties comparable to that of a standard drug, albendazole. Further studies are, therefore, needed to confirm the anthelmintic properties of these plants, particularly in *in vivo* studies. The result of this study may be

significant as the inhibition of egg hatch is possibly an important method of reducing pasture contamination by the animals during grazing helping in the overall helminth control programme.

Authors' contributions

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Writing – review & editing: I.K. Idika, M. Adamu.

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