NSAP 47th Annual Conference (JOS 2022) CONFERENCE PROCEEDINGS
THEME: SECURING ANIMAL AGRICULTURE AMIDST GLOBAL CHALLENGES

IN VITRO ANTHELMINTIC EFFICACY OF METHANOL STEM-BARK EXTRACT OF *ANOGEISSUS LEIOCARPUS* AGAINST *ASCARIDIA GALLI* INFECTION IN POULTRY

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ABSTRACT

Ascaridia galli is an important gastrointestinal nematode of birds that has been implicated as one of the *most common parasites of poultry. The main method of controlling nematode parasites of poultry has been through the use of synthetic anthelmintics. However, these drugs are scarcely available to poor farmers or rural communities. Anogeissus leiocarpus stem-bark methanol extract (AME) was investigated for its in vitro anthelmintic activity against Ascaridia galli. The extract was prepared by cold maceration and five different concentrations of the extract was studied (4 mg/ml, 2 mg/ml, 1 mg/ml, 0.5 mg/ml and 0.25 mg/ml). Albendazole and 0.1M sulphuric acid were used as positive and negative control respectively. The* IC_{50} *for the egg hatch assay was 0.25 mg/ml. There was no significant difference in the egg hatch inhibitory (EHI) activity of the extract when compared to albendazole. Higher concentrations of the extract had superior larvicidal activity. Anogeissus leiocarpus (A. leiocarpus) could find application in anthelmintic therapy in veterinary practice.*

Keyword: Anthelmintic, stem-bark, *Anogeissus leiocarpus*, *in vitro*, *Ascaridia galli*

INTRODUCTION

Helminths are multicellular worms that could either be free living or survive by feeding on a living host sometimes causing illness to the host (Hedley *et al*., 2015). *Ascaridia galli* is the most important parasite of poultry (Wongrak *et al*., 2014). Helminth infections lead to a marked loss in weight, decreased egg production and egg weight (Permin, 2020). Globally, the major control method employed against helminth parasites is the use of chemotherapy (Sargison, 2011). The cost of these drugs, poor availability and the development of resistance are some of the factors that have militated against the use of these drugs. A number of plant species have been used and reported in different parts of the world against nematode infections in animals and humans (Akhtar *et al*., 2020). *A. leiocarpus* is one of such plants.

MATERIALS AND METHODS

Plant material: The plant material was collected from Panhawya, Giwa local government area of Kaduna state. The flower, leaves and seeds of the plant were taken to the Herbarium, Department of Botany, Faculty of Life Sciences Ahmadu Bello University, Zaria, Nigeria, for identification and a voucher specimen No. 0900389 was deposited. The stem-bark was dried to constant weight in open air at room temperature for 3weeks. The dried sample was then ground into powder form.

Preparation of extract: 500 g of the powdered stem-bark was extracted by cold maceration using 98% methanol with agitation of the mixture at intervals over a period of 72 hours. A solvent to dry weight ratio of 5:1 was used. The extract was filtered into a beaker and then concentrated in an evaporating dish placed in an oven at 40^{o}C. The extract which yielded 11.52% (W/W) was then stored in a refrigerator.

Phytochemical screening: Phytochemical screening of AME was conducted to determine the presence of secondary metabolites using standard procedure as described by Evans (2009).

Ascaridia galli egg recovery: *Ascaridia galli* eggs were recovered from fresh intestinal scrapings of domestic fowls. The intestinal segments were opened to collect adult worms which were washed in physiological saline, then transferred to 0.5% KOH to dissolve attached tissues. Egg recovery was carried out using simple centrifugation technique (Prastowo & Ariyadi, 2015) with slight modifications. The adult worms were crushed into a paste, then washed in 0.5% KOH solution for 30 minutes. The preparation was then centrifuged at 1500rpm

for 3 minutes, then the supernatant decanted, then the eggs washed with distilled water. This was repeated thrice. 0.1% sulphuric acid (embryonating fluid) was then added to the eggs and then centrifuged twice at 1500rpm for 3 minutes. The eggs collected were suspended in the embryonating fluid and was estimated at 142 eggs per 0.1ml of the sample.

Egg hatch assay: Egg hatch inhibition (EHI) assay was done following the method of Agarwal (2019) with slight modifications. 0.07ml of the egg stock solution which contained about 100 eggs was measured into a 48-well microtitre plate. 1ml of the plant extract at various concentrations (4mg/ml, 2mg/ml, 1mg/ml, 0.5mg/ml, and 0.25mg/ml) were then added. 0.1% sulphuric acid was used as negative control in the last well, while Albendazole at various concentrations (1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml and 62.5 µg/ml) were used as positive control. The experiment was done in duplicate. The micro-titre plate was then incubated for two weeks at the end of which the hatched eggs in each well was recorded.

Larval mortality assay: *Ascaridia galli* eggs were cultured using the method of Agarwal, 2019 with few modifications as decribed for egg hatch assay. The egg stock solution was transferred into petri dishes and incubated for 3 weeks at 37^0c to develop to infective larvae (L2). The stock larval solution in the petri dishes was transferred into a beaker. 0.06ml which contained about 100 larvae was measured into a 48-well micro-titre plate. 1ml of the plant extract at the previous concentrations were then added to the wells. 0.1M sulphuric acid was the negative control, while Albendazole was the positive control. The micro-titre plate was incubated for 24 hours and evaluated, also at 48 and 72hours respectively. The number of dead larvae in each experimental well was recorded.

Statistical analysis: The best-fit IC50 value was calculated with 95% confidence interval. The EHI activity of the plant extract was further assessed by comparing with the egg hatch inhibitory activity of albendazole at 1 mg/ml concentrations by one-way ANOVA and Tukey's multiple comparison test using GraphPad Prism version 5.0 for Windows. The relation below gives the percentage egg hatch inhibition parameter:

$$\frac{\text{Mean No. of eggs hatched in the control well} - \text{Mean No of eggs hatched in the treated wells}}{\text{Mean No of eggs hatched in the control well}} \times 100$$

RESULTS

Phytochemical screening: The presence of saponins, anthraquinone, Flavonoids, alkaloids and tannins were detected in crude methanol stem-bark extract of *Anogeissus leiocarpus.*

Egg hatch inhibition: Crude methanol stem-bark extract of *A. leiocarpus* showed concentration-dependent anthelmintic activities in inhibiting egg hatching (figure 1). The IC_{50} is 0.25mg/ml for the plant extract, while IC_{50} of albendazole is 0.055mg/ml.

Larval mortality assay: The plant extract showed time-concentration-dependent anthelmintic activities in killing the L2s as shown in figure 2 below.

DISCUSSION

Phytochemical screening of the crude methanol stem bark extract of *Anogeissus leiocarpus* showed the presence of Saponins, Anthraquinones, Flavonoids, Alkaloids and Tannins and absence of carbohydrates. *Mann et al.* (2010) reported the presence of tannins, alkaloid, steroid, saponin and phenol. Similarly, Kabore *et al*. (2007), reported the presence of Flavonoids, Saponins and Tannins, from the stem bark methanol extract of *A. leiocarpus*. The IC50 obtained for the EHI assay of AME was 0.25 mg/ml. This is in line with the result obtained from the work of Ademola & Eloff (2011), in which the value was 0.219 mg/ml for the butanol fraction, but was different from 0.093 mg/ml for the chloroform fraction and 0.196 mg/ml for the water in methanol fraction of *A. leiocarpus* leaf extract respectively. The EHI activity of *A. leiocarpus* was comparable to that of the standard drug albendazole. The anthelmintic activity of the extract on L2 larvae was slightly higher than that of the standard drug albendazole. At 1 mg/ml, the plant extract killed 44% of the larvae after 72 hours, while Albendazole killed 41% of the larvae after 72 hours. The anthelmintic activity of the plant extract could be attributed to the phytochemical constituents present in the plant. Studies have demonstrated the anthelmintic

activity of tannins, flavonoids, and alkaloids (Wang *et al.,* 2010). Tannins have been shown to block ATP synthesis and they can also bind to free protein leading to nutrient starvation of the larvae (Jain *et al*., 2013).

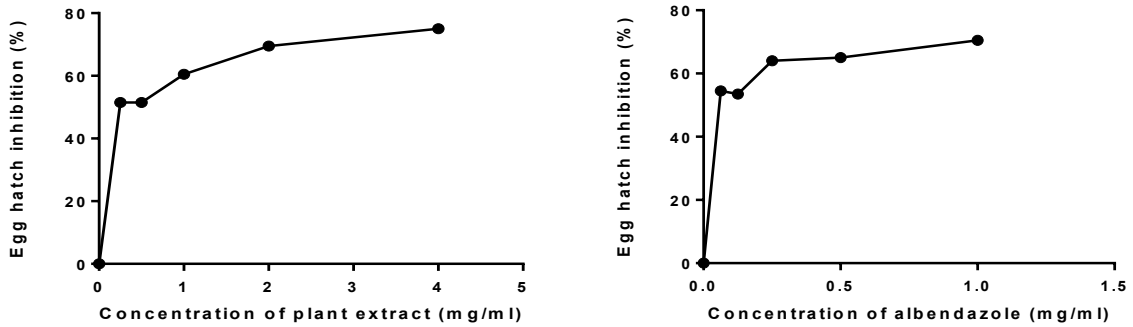

Figure 1: A non-linear regression graph of EHI assay of crude methanol stem-bark extract of *A. leiocarpus* at various concentrations.

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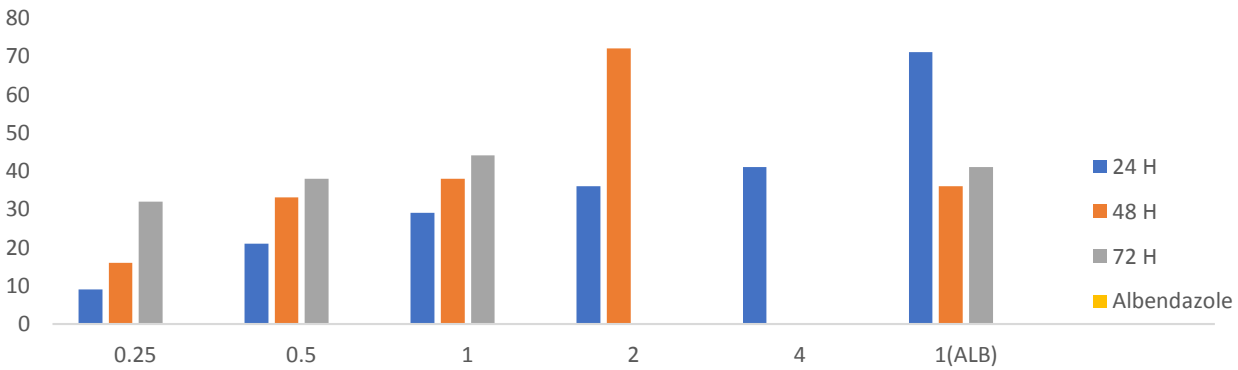

Figure 2 Results for Larval mortality assay of Crude methanol stem-bark extract of *A. leiocarpus* and albendazole at various concentrations and time intervals

CONCLUSION AND RECOMMENDATIONS

Methanol stem-bark extract of *A. leiocarpus* appears to possess some anthelmintic properties that supports its traditional use by farmers. Its activity is comparable to that of albendazole. However further *in vitro* and *in vivo* studies to evaluate its toxicity and toxic residue is recommended. Spectroscopic studies of the active principles and elucidation of their structure could provide leads for drug discovery.

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