INHIBITORY EFFECTS OF *ARTEMISIA ANNUA* METHANOL LEAF EXTRACTS ON SPORULATION OF *EIMERIA TENELLA* OOCYSTS OF CHICKEN

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ABSTRACT

Coccidiosis is a serious problem in poultry, and it is one of the major causes of economic loss in the poultry industry. The surge of drug- resistance in Eimeria parasites and also increasing global demands for organic meat products has necessitated the search for alternative treatment. The study was therefore aimed at evaluating the inhibitory effects of Artemisia annua methanol leaf extract (AME) on sporulation of Eimeria tenella of chicken in vitro. 90 g of ground Artemisia annua (A. annua) leaves were extracted with methanol through cold maceration method. Four different concentrations: 5 mg/ml, 10 mg/ml, 20 mg/ml and 40 mg/ml of AME was used to study its sporulation inhibitory effects against Eimeria tenella. Amprolium (15 mg/ml) served as the positive control, while potassium dichromate (K2Cr2O7) was used as the negative control. The extract showed a dose-dependent sporulation inhibition of Eimeria tenella. The highest sporulation inhibition of 100% was obtained at highest concentration of AME used in the study (40mg/ml). There was no statistically significant difference between sporulation inhibition produced by AME at 20 mg/ml and 40 mg/ml when compared to the standard drug Amprolium. This study showed that Artemisa annua methanol leaf extract possesses inhibitory effects on sporulation of Eimeria tenella and thus supports the traditional use of Artemisia annua as an anticoccidial.

Keywords: Anticoccidial, *Artemisia annua*, *Eimeria tenella*, Sporulation, Inhibition.

INTRODUCTION

Poultry holds a very important place in nutrition in developing countries as it is a source of high-quality protein and essential nutrients that is relatively affordable (Mottet and Tempio, 2017). Coccidiosis has been recognised as the most important intestinal disease of poultry of high economic impact (Attree *et al*., 2021). It is caused by the apicomplexan protozoan parasite belonging to the genus Eimeria (Kadykalo *et al*., 2018). Chemical prophylaxis and treatment have been the main method of controlling coccidiosis globally (Attree *et al*., 2021, However, in recent times there has been a spate of antimicrobial resistance globally, also widespread concerns pertaining the safety of anticoccidials and how they impact human, animal and environmental health has been generated (Mooney *et al*., 2020). This has led to the increased interest in screening medicinal plants as alternative anticoccidials. *Artemisia annua* is one of such plants (Drăgan *et al*., 2014).

MATERIALS AND METHODS

Plant material

The leaves of *Artemisia annua* were purchased from Amaru market, Zaria city, Kaduna state and submitted to the Herbarium at the Department of Botany, Faculty of Life Sciences. Ahmadu Bello University, Zaria, Nigeria, for identification. Sample number ABU0485 was assigned. The dried sample was ground into powder form.

Preparation of extract

Ninety grams 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 hrs. A solvent to dry weight ratio of 3:1 was used for the first 48 hrs, after which a solvent to dry weight ratio of 2:1 was used for rinsing. The extract was filtered into a beaker and then concentrated in an evaporating dish placed in an oven at 40^{o}C. The extract was then left standing at room temperature for two days to ensure complete evaporation of any remaining solvent. The extract which yielded 17.8% (W/W) was then stored in a refrigerator.

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

***Eimeria tenella* oocyst collection**.
Eimeria tenella oocyst were harvested from the caecae of infected chickens. The caecae were placed in a clean, air-tight container and transported to the Helminthology laboratory, Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine Ahmadu Bello University Zaria. The caeca were cut open longitudinally to collect the contents and scrapings, this was processed using simple flotation technique and the oocysts were then prepared in phosphate buffered solution. The oocyst harvest was enumerated microscopically and then adjusted to a concentration of 200 oocysts/ml in 2%w/v potassium dichromate in distilled water.

***In vitro* sporulation inhibition test**
The methanol leaf extract of *A. annua* was tested at 4 different concentrations: 5 mg/ml, 10 mg/ml, 20 mg/ml and 40 mg/ml, 2.5% potassium dichromate was used as the negative control, while amprolium 15 mg/ml was used as the positive control. The experiment was carried out in three replicates.Using a microtitre pipette, 200 unsporulated oocysts were transferred to each well of the microtitre plate. The extracts (40, 20, 10, 5mg/ml), Amprolium (15mg/ml) and 2.5 % potassium dichromate were added to the labelled microtitre plate. The plates were kept in an incubator at a temperature of 27◦ C with a relative humidity of 65% for a period of 48 hours, after which the oocysts were washed with physiological buffered saline (PBS) and viewed under the microscope for sporulation. Percentage sporulation inhibition of oocysts was calculated using the following formula:

$$\text{Sporulation (\%)} = \frac{number\ of sporulated\ oocyst}{total\ number\ of\ oocysts} \times 100$$

Statistical analysis
Results were subjected to one-way analysis of variance (ANOVA). Tukey's post hoc test (Graph Pad prism version 5.0) was used to test for differences in sporulation inhibition between the treatment groups. Values of $P \leq 0.05$ were considered significant.

RESULTS
Phytochemical screening
Phytochemical screening showed the presence of alkaloids, anthraquinones, carbohydrates, flavonoids, saponins, and phenols.

Sporulation inhibition Test
The percentage sporulation inhibition produced by *Artemisa annua* leaf extract was concentration dependent. Figure 1 represents the *in vitro* sporulation inhibition effects of *Artemisa annua* leaf extracts against *Eimeria tenella* oocysts. There was no significant difference between the sporulation inhibition produced by AME at 20 mg/ml and 40 mg/ml when compared to amprolium, but there was a significant ($p < 0.05$) increase in sporulation inhibition produced by amprolium and the extract at 20 mg/ml and 40 mg/ml concentration respectively when compared to extract at 5mg/ml and 10 mg/ml.effects on the sporulation of *E. tenella* oocysts ($p < 0.05$) compared to negative control.

DISCUSSION
Qualitative phytochemical screening showed the presence of alkaloids, anthraquinones, carbohydrates, flavonoids, saponins, tannins and phenols in the methanol leaf extract of *A. annua*. Ashok and Upadhyaya, (2013) reported the presence of carbohydrates, saponins, phytosterol, proteins and amino acid, tannin, phenolic compounds and flavonoids. The difference in the phytochemical compounds observed could be as a result of factors such as: difference in the solvent and extraction methods (Adam *et al*., 2019), genetics, environment and climate the plant was harvested from (Davey *et al*., 2009).

The *in vitro* sporulation inhibition activity of *Artemisia annua* leaf extracts against *Eimeria tenella* at 20 mg/ml (96.5%) and 40 mg/ml (100%) concentration was comparable to that of the standard drug amprolium (99.8%). Similar activity was reported by Maodoa *et al*. (2024) who recorded a 100% sporulation inhibition using 300 mg/ml methanol extract of *Artemisia sibieri* leaves. Fatemi *et al*. (2015)

recorded 31% inhibition using ethanol extract of *A. annua* at 5 mg/ml, which was similar to the 27.5% sporulation inhibition produced by AME at 5 mg/ml concentration. The sporulation inhibition activity of the plant extract could be attributed to the phytochemical constituents present in the plant. The exam mechanism of action is not well understood but it could be attributed to the ability of the phytochemical compounds to penetrate into the oocyst membrane and harm the sporont (Maodoa *et al*., 2024).

CONCLUSION

Artemisia annua methanol leaf extract (AME) showed very good sporulation inhibition effect on *Eimeria tenella* of chicken. Its activity is comparable to that of Amprolium and could serve as a potential alternative to chemotherapy. However, further studies *in vivo* to evaluate its efficacy and toxicity profile is recommended. Spectroscopic studies of the active compounds and elucidation of their structure could provide leads for drug discovery.

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