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Full Length Research Article

PROPHYLACTIC AND THERAPEUTIC STUDIES ON ETHANOLIC ROOT EXTRACT OF *ASPARAGUS AFRICANUS* AGAINST *EIMERIA* INFECTION IN BROILER CHICKS

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ABSTRACT
Poultry birds are commonly infected with *Eimeria* species, which causes economic losses in poultry farms. This study evaluated the *in-vivo* anti-coccidial activity of ethanolic root extract of *Asparagus africanus* in broiler chicks infected with *Eimeria* species. Phytochemical constituents and acute toxicity of the extract were determined. Prophylactic and therapeutic studies were carried out. In the prophylactic study, three groups of chicks were infected with about 250 *Eimeria* oocysts each and were orally-treated 2hr later with extract doses of 500mg/kg, 1000mg/kg, and 2000mg/kg respectively. Amprolium was used for positive control group, but one group was left untreated as negative control. In the therapeutic study, three groups of experimentally-infected broilers that had developed symptoms of coccidiosis were once daily for 5 days orally administered with 500mg/kg, 1000mg/kg and 2000mg/kg of the extract respectively. A positive control group was treated with Amprolium, while the negative control group was left untreated. Efficacy of the extract against *Emeria* was evaluated by severity of bloody-diarrhoea and number of oocysts/g of faeces. The ethanolic root extract contained various phytochemicals, which was safe at a high dose of 4000mg/kg. Bloody-diarrhoea was milder in both Amprolium-treated and extract-treated groups. Mean of oocysts/g of faeces in the untreated group ($P<0.0$) was significantly higher (4841 oocysts/g of faeces) compared to Amprolium-treated (1604 oocysts/g of faeces) and various extract-treated groups. The findings of this study suggest that root extract of *Asparagus africanus* has anti-coccidial activity, and can serve as an alternative means for controlling avian coccidiosis caused by *Emeria* species.

Keywords: *Asparagus africanus, Eimeria* species, Coccidiosis, Oocyst, Broiler chicks.

INTRODUCTION

Eimeria is an intracellular protozoa belonging to the phylum Apicomplexa and family Eimeriidae. *Eimeria* is responsible for coccidiosis in both birds and livestock (Fatoba *et al*., 2020; Qaid *et al*., 2022; El-Shall *et al*., 2022), but it is an economically important disease in poultry industry (Allen and Fetterer 2002; Muthamilselvan *et al*., 2016). Nine species of *Eimeria* have been identified in birds (Joyner and Long, 2008). Coccidiosis is one of the major parasitic diseases of poultry worldwide (Lee *et al.,* 2009). The parasite destroys the intestinal epithelium with consequent reduction in feed efficiency, body weight gain and egg production (Min *et al*., 2004; Dalloul and Lillehoj, 2005; Han *et al*., 2022). The disease is often characterized by marked morbidity, mortalities, as well as reductions in both productivity and feed conversion efficiency in affected chickens (Jang *et al.,* 2007; Hussain *et al.* 2021). Effective use of anti-coccidial drugs over the past 50 years partly provided basis for rapid growth of poultry industry and an increased availability of high quality poultry products to consumers. However, there has been an increase in resistance of avian coccidia to currently used anti-coccidial drugs (Williams, 2006; Han *et al*., 2022). Several compounds and extracts had been used in the treatment of coccidiosis including artemisinin (Arab *et al*., 2006), betaine (Klasing *et al.,* 2002; Fetterer *et al.,* 2003), root preparation of *Echinacea purpurea* (Allen, 2003), mushrooms and their extracts (Borchers *et al.,* 2004; Guo *et al.,* 2004; Guo *et al.,* 2005; Dalloul *et al.,* 2006), essential oils of *Origanum vulgare* (Giannenas *et al.,* 2003) among many others. Plant extracts and essential oils are relatively environmentally safe, producer- and consumer-friendly. They have been effective against many illnesses (Han *et al*., 2022). Since *Asparagus africanus* are abundant in most parts of Nigeria, this study was focused on harnessing root extract of this plant as local treatment against coccidiosis in broiler chicks.

MATERIALS AND METHODS

Plant materials

The root of *Asparagus africanus* was collected from a bush around Tudun Wada in Jos North Local Government Area of Plateau State, Nigeria. The plant was authenticated at Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University Zaria, where a voucher number (V/No: 3250) was deposited. The roots were air-dried under shade until they became well dried before they were ground to powder using mortar and pestle.

Ethanolic extraction and phytochemical screening

From the powdered specimen, 450g was weighed and divided into three bottles, then 750ml of ethanol was added to each, stoppered and shaken vigorously before placing them on a bench for 48hr. The sample was filtered using a double-layered cheesecloth and the filtrate was concentrated on a water bath at 60˚C overnight. After 72hr, golden brownish residue weighing 106g was obtained. The residue obtained from the ethanolic extraction was further subjected to phytochemical screening as described by Sofowora (1984) at the Toxicology unit in Department of Biochemistry, National Veterinary Research Institute, Vom, Plateau State, Nigeria.

Experimental animals

A total of 34 two-weeks old broilers chicks sourced within Zaria

were used for the experiment. The chicks were placed in a large room under controlled warmth and ventilation, fed and observed for one week to acclimatize before the commencement of the tests.

Collection, purification and culture of *Eimeria* oocyst

Eimeria oocyst used in this study were obtained from mature broilers slaughtered at an abattoir in Sabon-Gari, Zaria, Kaduna State, and from broilers diagnosed with coccidial infection at Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The gastrointestinal tract of the infected birds was carefully spread on a tray and only the caeca were obtained. The caeca were cut-open longitudinally and the contents were washed unto a beaker using distilled water. The washing was centrifuged, and the supernatant was discarded using a Pasture pipette. The sediment was remixed with saturated sodium chloride solution then allowed to stay undisturbed for 7 minutes in order for the oocysts float. The oocysts were harvested unto another tube. The traces of salt and colouring matter were removed by washing the harvested oocysts with distilled water several times through the process of centrifugation. Thereafter, the sediment containing the oocysts was divided into Petri dishes and sporulated by adding 2% potassium dichromate then left on the bench for 72hr. After sporulation, potassium dichromate was washed off several times using distilled water and centrifugation. The whole process was repeated after two days for a period of three weeks in order to get sufficient oocysts. Finally, all sporulated oocysts were pooled together, re-concentrated by centrifugation, and the final concentration of the oocysts was determined. Suspension of the oocysts was stored at 5^{o}C for further use.

Acute toxicity test of ethanolic root extract of *Asparagus africanus*

Twelve (12) day-old chicks (sourced at Zartech farms LTD) were brooded for 3-weeks before the commencement of the test. The chicks were divided into six groups A-F consisting of two birds each for the determination of acute toxicity of the ethanolic root extract of *Asparagus africanus.* The chicks from group A-E were treated orally with a graded dose of the extract (100, 200, 500, 1000, 2000 and 4000mg/kg of body weight. Those in group F (which served as control) were given only distilled water equivalent to the largest volume of extract administered by the same route. The birds were monitored for a period of 24hr for signs of toxicity and death. The birds were sacrificed after 24 hours and examined for necropsy for lesions.

***In- vivo* anti-coccidial effect of the *Asparagus africanus* root extract**

Two kinds of studies were carried out: a prophylactic/preventive study and a therapeutic study using 10 broiler chicks for each.

For the prophylactic study, five groups (A, B, C, D, and E) of two broiler chicks each were used. The birds in all the groups were infected with about 250 *Eimeria* oocysts each. Two hours after infection, oral treatments with extract doses of 500mg/kg, 1000mg/kg, and 2000mg/kg were administered to groups A, B, and C respectively; while D was treated orally with a preventive dose of Amprolium via drinking water. Group E was left untreated. All were observed for 16 days from the first day of infection. Oocysts shed in their faecal matter were determined using McMaster method.

For the therapeutic (treatment) study, five groups consisting of I, II, III, IV and V were infected with about 250 *Eimeria* oocysts and allowed to develop symptoms of coccidiosis before the commencement of treatment. Group I, II and III were treated orally with graded doses of the extract (500mg/kg, 1000mg/kg, and 2000mg/kg respectively). Group IV was treated with Amprolium in drinking water; while group V was left untreated. Weight of each chick was measured daily. Treatment commenced on the 6th day of post-infection, and continued daily until the 16th day. Oocysts shed in their faecal matter were determined using McMaster method.

Statistical Analysis

Data on *Eimeria* oocysts count were subjected to One-way analysis of variance (ANOVA) with Duncan post-hoc at 95% confidence interval using on IBM SPSS version 23.

RESULTS

Phytochemical analysis of *Asparagus africanus* revealed high presence of alkaloids, carbohydrates, cardiac glycosides, flavonoids and tannins. Saponnins were moderately present, while oxalates and anthraquinone were mildly present. However, phytates were absent (Table 1).

Symptoms of coccidiosis were noticed after the 4th day of infection (Table 2), but were well-established on the 6th day following analysis of oocyst count of their faecal matter. There were no signs of toxicity (such as depression, limping, drooping, anorexia or death following the administration of graded single doses of the *Asparagus africanus* extract from 100mg/kg to 4000mg/kg. There were also no lesions on their gastro-intestinal tract (Table 3).

From the bar chat (Figure 1), there was a gradual reduction in the number of faecal oocyst shed from the 8th day of post infection till the 16th day.

There was a gradual drop in body weight from 3rd day after infection. Even after the commencement of treatment on the 6th day of infection, there was drop in body weight. By the 9th day, a gradual increase was noticed in all the groups treated with Amprolium and *A. africanus* root extract. For the untreated group (negative control) body weight kept on decreasing (Table 3).

Table 1: Phytochemical constituents of *Asparagus africanus*

Phytochemicals	Inference
Alkaloids	+++
Anthraquinone	+
Carbohydrates	+++
Cardiac glycosides	+++
Flavonoids	+++
Oxalates	+
Phytates	-
Saponins	++
Tannins	+++

Keys: Negative (-); Mild (+); Moderate (++); High (+++)

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Table 2: Evidence of coccidiosis in experimental broiler chicks

Day	Bloody-diarrhoea	Depression	Ruffled feather	Downward-hanging wings
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	+	+	-	-
5	+	+	-	-
6	++	++	+	+
7	+++	+++	+	+
8	+++	+++	++	++

Keys: Negative (-); Mild (+); Moderate (++); High (+++)

Table 3: Toxicity test of ethanolic extract of *Asparagus africanus* in experimental broiler chicks

Concentration (mg/kg)	Depression	Limping	Drooping	Anorexia	Death	Intestinal lesions
100	-	-	-	-	-	-
500	-	-	-	-	-	-
1000	-	-	-	-	-	-
2000	-	-	-	-	-	-
3000	-	-	-	-	-	-
4000	-	-	-	-	-	-

Key: Negative (-)

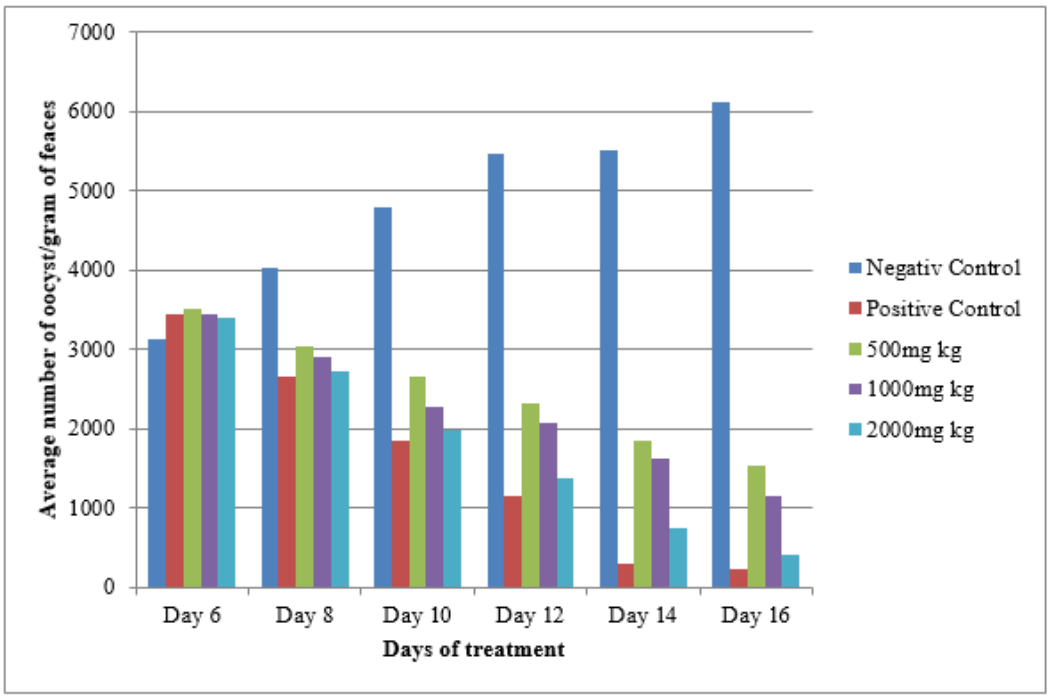

Figure 1: Prophylactic effects of ethanolic root extract of *Asparagus africanus* in the broiler chicks (P<0.05)

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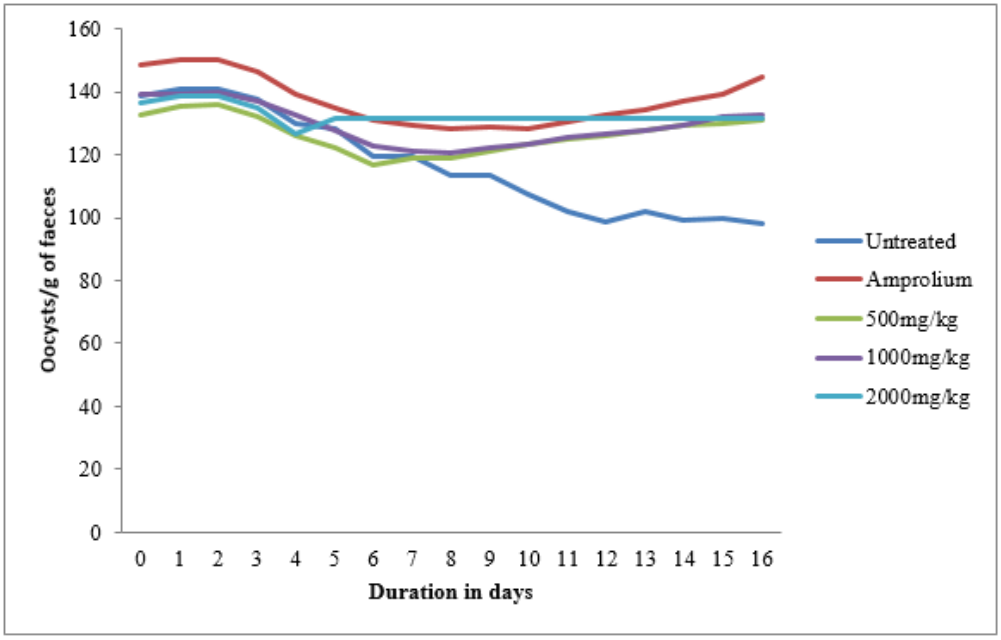

Figure 2: Therapeutic activity of ethanolic root extract of *Asparagus africanus* in broiler chicks with coccidiosis (P<0.05).

Table 4: Average weight of broiler chicks during the period of the experiment

Duration (Days)	Negative Control Weight (g)	Positive Control Weight (g)	500mg/kg Weight (g)	1000mg/kg Weight (g)	2000mg/kg Weight (g)
0	138.5	148.5	132.5	139	136.5
1	141	150	135.5	139.5	138.5
2	141	150	136	140	138.5
3	137.5	146.5	132	137	135
4	130	139	126	132.5	126.5
5	128	135	122.5	127.5	124.5
6	119.5	131	117	123	120
7	119.5	129.5	119	121	119.5
8	113.5	128.5	119	120.5	118.5
9	113.5	129	121	122.5	119.5
10	107.5	128.5	123.5	123.55	121.5
11	102	130.5	125	125.5	124.5
12	98.5	132.5	126	126.5	125.5
13	102	134.5	127.5	127.5	127.5
14	99	137	129.5	129.5	128.5
15	100	139.5	130	132	132
16	98	144.5	131	132.5	131.5

DISCUSSION

Poultry industry in Nigeria has greatly expanded due to high demand, but it faces severe threats due to infections of parasitic, bacterial and viral origins. Hence, it is imperative to source for local remedies by mainly harnessing our native plant-derived sources (like *Asparagus africanus*) that are safe and without any tissue-residual effects or reported drug resistance. Coccidiosis due to *Eimeria* species in poultry birds affects their growth, with consequent reduction in economic value. These parasites have demonstrated resistance to conventional prophylactic drugs in animal feeds (Qaid *et al*., 2022). New approaches to remedy coccidiosis have adopted the use of natural products, probiotics, live vaccines, improved farm management practices, and modulation of the chicken immune system (Allen and Fetterer,

2002; Dalloul and Lillehoj, 2005). However, continued emergence of drug resistance, severe allergic reactions to vaccines and the advocacy for supply of residue-free meat, has necessitated the search for better alternatives that employ the use of plant-derived phytochemicals (Chapman *et al.*, 2010; Muthamilselvan *et al.*, 2016; Upadhaya *et al.*, 2019; Qaid, *et al.*, 2022; El-Shall *et al.*, 2022).

Presence of flavonoids, saponins and tannins are indicative that *Asparagus africanus* plant is rich in antioxidants. This is why many studies had alternatively applied plants with rich antioxidant content in the treatment of coccidiosis (Batool *et al.,* 2019; Hussain *et al.*, 2021).The ethanolic extract of *Asparagus africanus* was found to be non-toxic on the broiler chicks even at very high concentration of 4000mk/kg. This plant is abundant in Nigeria, which will present cheap alternative to combat *Eimeria* infections in poultry birds. The plant is known to contain some bioactive compounds like pyrodextrin and inulin (El-Shall *et al.*, 2022).

The *Eimeria*-infected birds in this study showed evidence of bloody diarrheoa, depression, ruffled feathers and wings that hung downward. When *Eimeria* parasites destroy intestinal epithelial cells in the caecum, which is one of the most important parts of the digestive system, it leads to low body weight in birds (Lan *et al.*, 2016; Qaid *et al.*, 2022). Amprolium is widely used to control coccidan infections. However, extract of *Asparagus africanus* demonstrated prophylactic and curative efficacies in the experimental broiler groups at different doses. Therefore, proper poultry management practices and timely application of anticoccidial agents will help to control the infection (Godwin and Morgan, 2015; Fatoba *et al.*, 2020). Aside poultry birds, *Eimeria* species affect other farm animals like rabbits (Mailafia *et al.*, 2010). This has necessitated researchers into seeking for plant-derived solutions against coccidiosis. For example, Murshed *et al.* (2023) worked on *Calotropis procera* leaf against *Eimeria stiedae* in rabbits, which had inhibitory effect. Also, extract of *Cinnamomum verum* bark had been shown to improve feed conversion ratio in broiler chickens infected with *Eimeria tenella* (Qaid *et al.*, 2022).

Conclusion

Coccidiosis due to *Eimeria* species affects broiler birds. This study revealed prophylactic and therapeutic potentials of ethanolic extract of *Asparagus africanus* against *Eimeria* species in broiler chicks. The ethanolic extract was found to be no-toxic and helped the experimentally-infected birds to steadily regain body weight.

Conflict of interest

The authors wish to declare that there is no any conflict of interest in this study.

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