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Evaluation of the Medicinal Potentials of Soursop (*Annona muricata* Safford) Leaf Extracts against *Eimeria tenella* Infection in Broiler Chickens

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Authors' contributions

This work was carried out in collaboration among all authors. Author IBO did the conceptualization, supervision and editing, Authors SOA and COD did the methodology, reviewing, formal analysis, writing and editing. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Poultry industry in Nigeria has recorded considerable expansion in recent times but not without poultry diseases as one of the major threats to boosting poultry production. Protozoa cause the majority of parasitic infections and cause high mortalities. Parasitic diseases such as coccidiosis are of particular importance because of their high incidence in poultry occasioned by the tropical environmental conditions under which the farmer operates. The resistance to common anticoccidial drugs has led to exploring other natural sources for prophylaxis and therapy. This research evaluated the therapeutic efficacies of *Annona muricata* at different dosages of 100, 125, 165mg/kg body weight using 120 broilers at 4 weeks of age.

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Place and Duration of Study: Animal Parasitology and Microbiology Research Unit, Department of Animal Production and Health, Federal University of Technology, Akure, Nigeria, between September and December, 2022.
Methodology: 120 broilers of 4 weeks old were used for this study using completely randomized study design. The birds were infected with sporulated *Eimeria tenella* oocysts at week 4. Treatment with ethanolic extract of *Annona muricata* was administered orally at week 5 using different dosages of 100, 125, 165mg/kg body weight for five consecutive days. Treatment commenced after the isolation of *Eimeria* oocysts in the faeces and onset on coccidial clinical signs as parameters to confirm manifestation of the infection. Blood parameters, faecal oocysts count and biochemical assays were conducted.
Results: Results revealed that therapeutic administration of *Annona muricata* leaf extracts at minimum dose 100mg/kg body weight had significant reduction ($P<0.05$) on excreted *Eimeria* oocysts counts without any significant deviation from normal haematological and biochemical indices of the broilers.
Conclusion: This study suggests the use of *Annona muricata* in the treatment of coccidiosis in broiler chickens. It is therefore recommended that its extracts be further explored for actual phytochemical constituents responsible for the displayed anticoccidial properties.

Keywords: Annona muricata; anticoccidial activities; Eimeria tenella; faecal oocysts; blood parameter; biochemical assay.

1. INTRODUCTION

Nowadays, poultry production has high demand all over the world. Poultry industry in Nigeria has recorded considerable expansion in recent times but not without poultry diseases as one of the major threats to boosting poultry production. This increasing demand has led to the usage of numerous antibiotic-free products. There is an increased pressure to reduce the number of antibiotics that are used as bacteriostatic or bactericidal agents for poultry, so there is a crucial requisite for unconventional resolutions to sustain the productivity and efficiency of poultry [1]. Now, there is also the use of herbal plants as an alternate for the prevention of intestinal parasitosis [2], [3].

Protozoa and helminths cause the majority of parasitic infections and cause high mortalities [4]. Parasitic diseases such as Coccidiosis are of particular importance because of their high incidence in poultry occasioned by the tropical environmental conditions under which the farmer operates [5]. Of all nine *Eimeria* species identified in poultry [6], *Eimeria tenella* and *Eimeria maxima* are the most common [7], causing tissue damage, lowered feed intake, poor absorption of nutrients from the feed, dehydration and blood loss [5].

Vaccines as well as chemotherapeutic agents have been employed to suppress the menace of coccidiosis in the past, but are expensive to produce, can lose their potencies over a long storage period, resident organisms can undergo genetic shift/drift making the use of vaccine unreliable. The reduction in the use of chemically manufactured drugs can be attributed to poverty, inaccessibility, and decaying infrastructure. Also, resistance to same drugs over a period of time is a risk factor [8]. The use of alternative medicine, as a result, has been advocated [4]. Several diseases can be cured using traditional medicines that utilize plant, herb, or mineral ingredients [9].

Hence, the need for this research to explore the abundance of plants to provide surplus remedies against the havoc posed by coccidiosis to broiler farming in Nigeria. *Annona muricata* is identified candidates to be explored for possible remedy to coccidiosis menace in the poultry industry.

Soursop (*Annona muricata*) is endemic to the warmest areas of the tropics of South and Central America and the Caribbean. It is now widely found in tropical and subtropical regions of Central and South America, Western Africa, and Southeast Asia [10]. The aqueous extract of *A. muricata* is used to control lepidopteran larvae, aphids and thrips, among others [11], [12], [13]. Also, the leaves, bark, and roots of *A. muricata* have been used for its anti-inflammatory, hypotensive, sedative, hypoglycemic, smooth muscle relaxant, antiplasmodic and antitrypanosomal effects [14], [15], [16] among other uses.

2. METHODOLOGY

2.1 Test Organisms

Typed *Eimeria tenella* was obtained from Nigeria Veterinary Research Institute, (NVRI) Vom, Jos, Ngeria.

2.2 Identification and Authentication of Plants

The leaves of *Annona muricata* were collected from their natural habitats around The Federal University of Technology, Akure, Ondo State and proper plant identifications was done by the Department of Pharmacognosy, Faculty of pharmacy, Obafemi Awolowo University, Ile Ife with voucher number FPI 2402.

2.3 Crude Extraction of *Annona muricata*

The plants leaves were dried at room temperature, then pulverized with electric hammer mill. 1000g of the fine powder was soaked in 5liters of ethanol for 76hours. The extract was filtered using muslin cloth and filtrate was concentrated to dryness using Rotary-evaporator. The resulting organic extracts was stored in sterile bottles at refrigeration temperature (4 -8°C) before carrying out the in vivo activities.

2.4 Phytochemical Analysis of Plants' Crude Extract

The powdered crude extracts of the plants leaves were subjected to phytochemical analysis for the presence of plant secondary metabolites using standard qualitative procedures [17], [18], [19].

Determination of LD_{50}: Lorkes' method as described by Enegide et al*.,* [20] was used to determine the Lethal Dose50 (LD_{50}) from which the graded doses were calculated. Determination of graded doses: No mortality was recorded in any of the birds of all the dose variations, so the random starting dose was picked as 100mg/kg with the other doses as 125mg/kg and 165mg/kg.

2.5 Experimental Design

A hundred and twenty (150) broiler chickens were purchased from a reputable hatchery and reared as a single group from day old to fourteen (14) dayswith appropriate vaccination/medication schedules. Certified commercial feed but without anticoccidial agents of any kind was served *ad libitum*.

2.6 Infection and Monitoring

Each bird was infected with 0.2 ml of a suspension of sporulated *Eimeria tenella* containing about 50,000 viable oocysts per millilitre as illustrated by Gotep et al*.,* [21].

After confirmation of the disease manifestation, the broilers were distributed into 9 groups consisting 15 birds each. Extract was administered at 100mg/kg, 125mg/kg and 165mg/kg to group 1-3. Groups 4, 5 and 6 were the negative control, uninfected untreated and positive control groups respectively. Each treatment group were treated with the plant extracts, once a day for five (5) days per *os*.

Blood samples were collected for haematology and serum biochemistry. The intestine (caecum) and liver were harvested for histological examination. All collated data from the study were subjected to Analysis of Variance (ANOVA) using the SPSS version 22.

3. RESULTS AND DISCUSSION

Coccidiosis is a major source of concern to most especially poultry farmers around the world due to its difficulty to control, high cost of treatment and high risk of mortality following carcass emaciation and immunosuppression. Infections with species of the genus *Eimeria*, the causative agent of coccidiosis in poultry has been shown to be due to *Eimeria tenella*, *Eimeria necatrix*, *Eimeria brunette* and *Eimeria acervulina* in Nigeria [22].

Chemoprophylaxis and anticoccidial feed additives have been used in managing coccidiosis but emergence of drug resistant strains due to indiscriminate anti-coccidial drugs use [22] has complicated the management of coccidiosis on farms.

Totravet drug proved to be most effective in the treatment of coccidiosis among all drugs used because it treated the largest percentage of infected birds [7].

In this research, the Lethal Dose 50 (LD_{50}), efficacy parameters, haematological effect, liver function and phytochemical tests of the leaves are extrapolated. Infected birds displayed various clinical signs such as nutrients blood in faeces, anorexia, impaired body weight gain, and in some cases, mortality.

Table 1. Therapeutic effects of *A. muricata* leaf extracts on the Macmaster oocysts count of broiler chickens infected with *Eimeria* oocysts

	Pre-infection (wk 4)	Post-infection (wk 5)	Post-treated (wk 7)	% change from 5th week to 7th week
AnM100	175.00±0.00	718.75±44.19^{b}	200.00±17.68^{a}	-60.23±0.32^{a}
AnM125	200.00±70.71	931.25±397.75^{bc}	360.25±26.52^{a}	-52.58±14.84^{a}
AnM165	200.00±35.36	843.75±61.87^{bc}	300.25±8.84^{a}	-59.60±3.94^{a}
NiF	137.50±53.03	131.25±8.84^{a}	127.00±0.00^{a}	-3.10±0.00^{a}
INT	112.50±17.68	1368.75±150.26^{c}	1400.34±471.41^{b}	11.93±46.61^{b}
ITT	112.50±35.36	712.50±88.39^{b}	210.25±26.52^{a}	-71.60±7.38^{a}

Means on the same column with different superscripts are significantly different (P<0.05) Percentage change in oocysts count between period of infection (week 5) and period of treatment indicates reduction in oocysts due to the extracts administered. Though without significant differences along the column, the control drug had the highest percentage reduction (70%) while the two plants extracts posed same efficacies.

KEY

AnM100	*Annona muricata extract at 100mg/kg*
AnM125	*Annona muricata extract at 125mg/kg*
AnM165	*Annona muricata extract at 165mg/kg*
NiF	*uninfected untreated*
INT	*infected untreated*
ITT	*infected and treated with toltrazuril at 10mg/kg*

Phytochemicals analysis reveals that *Annona muricata* both possesses tannins, flavonoids, phenolics, saponins. The presence of these phytochemicals in *Annona muricata* has explored and utilised in them as antistress, anticancer agents, antifungi [23], antibacterial [24]. These phytochemicals present are believed to reduce the oxidative stress posed by the *Eimeria* organism.

The Lethal Dose 50 carried out following guidelines as laid down by OECD showed that *Annona muricata* has no toxic effects on the birds at 2500mg/kg body. Hence, it can be considered non-toxic and safe.

Compared with the uninfected group with decreased percentage oocysts count of (-51.00%), no significant difference was observed with *Annona muricata* extract-100mg/kg, 125mg/kg, 165mg/kg and the control drug (toltrazuril) groups. Whereas, infected untreated birds had an increased percentage OPG count of 11.93%, compared with the initial OPG.

This implies that the oocysts population available for continuation of the *Eimeria* life cycle had been reduced by the plants extracts but unhindered in the infected untreated group, though, numerically, the toltrazuril group, with decreased %OPG of -70.60 was more effective in cutting down the *Eimeria spp* population. Hence, the effectiveness of extract can be said to be dose dependent.

3.1 Haematological Parameters

The coccidia agent had a deteriorative effect on the PCV, HB, RBC, heterophil, the basophil, monocyte, lymphocyte and eosinophil. Post infection, there was a significant decrease in the PCV of all the treatment groups compared to the Uninfected (control) group associated with caecal haemorrhage following *Eimeria* attack as reported by Akhtar, [25].

The Red Blood Cell count, PCV, and Hb decreased post infection but improved post treatment with *Annona muricata* extract. Post treatment, the RBC count reflected no significant difference between the treatment groups and the uninfected groups. This infers that the previous sources of blood loss, caecal haemorrhage has been amended by the plant extract.

Similar to the observations of Akhtar [25] and Davies et al*.,* [26], the post-infection heterophil population of every other group was significantly higher than that of the uninfected groups indicative of infestation's immunosuppressing activities.

Post infection and post treatment, a significantly high level of eosinophils was recorded in the infected untreated groups. This is indicative of reduction in the level of damage done by the Eimeria organism.

Table 2. Percentage change in haematological indices

	PCV(%)	HB CONC(g/l)	RBC COUNT(10^{12}/l)	BASOPHILS (%)	HETEROPHILS (%)	MONOCYTES (%)	LYMPHOCYTES (%)	EOSINOPHIL (%)
AnM100	21.74±6.15^{abc}	16.05±9.00^{a}	92.39±81.47^{a}	-78.41±4.82^{a}	-21.15±0.00^{a}	-85.00±2.36^{a}	-38.10±6.74^{a}	-100.00±0.00^{a}
AnM125	6.55±2.52^{ab}	17.47±11.24^{a}	36.71±44.84^{a}	-70.84±5.89^{a}	-7.32±4.80^{a}	-58.59±20.00^{abc}	-16.67±0.00^{ab}	-95.42±0.59^{a}
AnM165	12.70±11.81^{ab}	7.64±8.78^{a}	76.32±33.50^{a}	-77.09±14.73^{a}	136.67±146.14^{a}	-56.82±9.64^{abc}	-52.57±19.95^{a}	-96.67±4.72^{a}
NiF	28.03±7.50^{bc}	3.75±3.01^{a}	63.90±26.09^{a}	-69.05±3.37^{a}	-18.73±9.51^{a}	-64.11±3.63^{abc}	-47.78±11.00^{a}	-97.62±3.37^{a}
INT	0.00±7.07^{a}	19.34±61.03^{a}	-16.56 ±16.98^{a}	0.00±0.00^{b}	5.60±13.71^{a}	-28.28±24.28^{bc}	-15.00±7.07^{ab}	-14.53±13.12^{a}
ITT	1.79±2.52^{a}	-0.53±3.54^{a}	1.35±5.35^{a}	0.00±0.00^{b}	-6.55±3.60^{a}	-20.00±28.28^{c}	29.17±5.90^{b}	150.00±212.13^{b}

Values on the same column with different superscripts are significantly different (P<0.05)

Table 3. Percentage change in biochemical indices

	AST(IU/L)	ALT (IU/L)	ALP (IU/L)	TP(g/dl)
AnM100	-65.51±54.43^{b}	-14.53±56.22^{a}	-42.76±17.18^{a}	125.54±81.74^{b}
AnM125	-89.94±24.34^{ab}	-20.15±12.68^{a}	-44.57±7.69^{a}	151.31±5.22^{b}
AnM165	-92.71±33.88^{ab}	-21.24±16.84^{a}	-35.96±0.35^{a}	164.62±6.53^{b}
NiF	-114.80±93.55^{a}	-37.55±16.33^{a}	8.81±4.12^{b}	143.33±61.28^{b}
INT	50.33±8.95^{c}	10.18±32.87^{b}	3.84±27.22^{b}	-6.72±25.99^{a}
ITT	-30.66±5.79^{b}	-17.18±30.67^{a}	-20.64±21.02^{a}	5.92±20.62^{ab}

Values on the same column with different superscripts are significantly different (P<0.05)

In this experiment, infested broilers with coccidian parasites showed highly significant increase in serum ALT and serum AST level as compared with control group (NiF) which is line with Patra et al., [27] and Mondal et al., [28] who reported that liver function test of the infected broiler chicken with Eimeria spp. showed a significant increase in the serum ALT, AST. This is suggestive that a significant assault was inflicted on the cell lining of the caecal wall and their inflammation is seen from the increased and severe blood loss causing tissue loss from the body may attribute to increased AST activity.

The highest reduction rates were noticed in AnM 165, -107% and -89% of each plants respective and this translates to the absence of as many damaged tissues (intestinal wall) as there was in the post-infection stage. It can also be said that the extracts helping in facilitating ulcer healing.

Haemogram of the pre-extract, post-extract administration and Post-infection stages reflected that all the haematological indices, except the eosinophils at the post-infection stage, had no significant differences when compared with the control, the NiF group.

The eosinophil levels, at the post-infection stage, are significantly highest in the AnM 100 and least in AnM 125. The lower the value of the eosinophil population, the more efficiency the extracts in ameliorating the detrimental effects of the coccidial organism. From this result, it suggests that the plants extracts, *Annona muricata* 125mg/kg (AnM 125) had the highest anticoccidial potentials of the doses in use.

4. CONCLUSION

Treatment with Annona muricata decreased percentage oocysts count, improved the Red Blood Cell count, PCV, and Hb while increasing serum ALT and serum AST levels in the extract treatment groups compared with the untreated. Based on this result and characteristics displayed by the *Annona muricata* extract, the plant can be said to possess anticoccidial property. Further purification, isolation and identification of bio active component from the plant is therefore recommended.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All of the animals used in this study received humane care according to the criteria outlined in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy Science and published by the National Institute of Health (USA). The ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animals' welfare during experiments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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