IRASS Journal of Applied Medical and Pharmaceutical Sciences
Abbriviate Title- IRASS J App Med Pharm Sci
ISSN *(Online) 3049-0901*
https://irasspublisher.com/journal-details/IRASSJAMPS
Vol-2, Iss-5(May-2025)

ANTICOCCIDIAL EFFICACY OF *VERNONIA AMYGDALINA* DEL. METHANOLIC LEAF EXTRACT AND ITS MAJOR FRACTIONS AGAINST EXPERIMENTAL *EIMERIA TENELLA* CHALLENGE IN BROILER CHICKENS

Markus Bukar Biallah[1]*, Oluyinka Oluseyi Okubanjo[2], Paul Ayuba Abdu[3], David Omagbe Oshadu[1], Paul Davou Kaze[1], Goni Abraham Dogo[1]

[*1] Department of Veterinary Parasitology and Entomology, University of Jos, Jos, Nigeria

[2] Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, Nigeria

[3] Department of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria

Corresponding Author **Markus Bukar Biallah**

Department of Veterinary Parasitology and Entomology, University of Jos, Jos, Nigeria

Article History

Received: 12 / 05 / 2025

Accepted: 27 / 05 / 2025

Published: 31 / 05 / 2025

Abstract: Coccididiosis is responsible for significant production losses due to mortality and weight loss in chickens. The traditional control using chemotherapy and vaccinations has suffered setbacks, prompting the search for alternative measures. Plant preparations are being researched for alternative and sustainable control methods of. Some trials have been conducted on the efficacy of some herbal products, and many have shown measurable efficacy in the prevention of coccidiosis. Plants of the genus *Vernonia* are widely used in ethnomedicine and ethnoveterinary practice. There are documents on the antiparasitic efficacy of *Vernonia amygdalina* extracts, especially in the control of apicomplexan protozoa. In this study, the protective efficacy *V. amygdalina* and its fractions was evaluated based on clinical signs, mortality, faecal and caecal scores, faecal oocyst output, and production performance against the standard treatment with amprolium. The experiment was carried out in a randomized complete block design where 210 broilers with similar body weight were assigned to seven experimental groups designated A-G, represented by two replicates of 15 birds each, and 100,000 of sporulated oocysts were administered *per os* on the 21 d of age except group A which served as negative control. Groups B and C, treated with normal saline and amprolium 125 mg/kg, served as positive (PC) and standard controls (SC), respectively. Birds were treated with methanolic extract (group D) 1,000 mg/kg; hexane, aqueous, and butanol fractions (groups E-G, respectively) at 500 mg/kg each, from 1 day before infection through 5 day post-infection (pi). All treatments were administered *per os.* It was observed that all treatments demonstrated good anticoccidial efficacy in the experimental chickens. There was a reduction in the severity of the clinical signs associated with coccidiosis in birds treated with methanolic extracts and their fractions (groups D-G). The methanolic extracts and their fractions prevented mortality (0%) compared with that (33.3%) recorded in the group administered with placebo (group B). Faecal score drastically reduced in birds treated with methanolic extract and its fractions, with the butanol fraction recording the lowest (1.00) by 6 dpi. By the end of the experiment, faecal scores returned to normal except for the positive control group. Caeca lesion score was significantly (p<0.05) higher in the PC group (3.00) and lowest in the methanolic extract and butanol fraction groups (1.00). Oocyst output (oocyst per gram of faeces, opg) was significantly high (p<0.05) in chickens treated with butanol fractions (763 opg) compared with the positive control group (125,213 opg). Additionally, feed intake, weight gain, and feed conversion ratio in chickens treated with methanolic extracts and their fractions were significantly better than the PC group by the end of the experiment. It can therefore be concluded that methanolic extract of *Vernonia amygdalina* and its fractions have profound anticoccidial activities in broiler chicken experimentally infected with *Eimeria tenella.*

Keywords: *Vernonia amygdalina,* leaf extract, *Eimeria tenella,* anticoccidial, broiler chicken

How to Cite: Biallah, M. B., Okubanjo, O. O., Abdu, P. A., Oshadu, D. O., Kaze, P. D., Dogo, G. A., (2025). ANTICOCCIDIAL EFFICACY OF VERNONIA AMYGDALINA DEL. METHANOLIC LEAF EXTRACT AND ITS MAJOR FRACTIONS AGAINST EXPERIMENTAL EIMERIA TENELLA CHALLENGE IN BROILER CHICKENS. *IRASS Journal of Applied Medical and Pharmaceutical Sciences, 2(5), 5-13.*

Introduction

In Nigeria and other developing countries in Africa and Asia, the poultry industry occupies an important position in the provision of animal protein to man. Worldwide, poultry meat represents 28% of the meat supply compared with 26.5% for beef [1]. Poultry diseases remain one of the major threats to boosting poultry production in Nigeria[2]. *Coccidiosis* is a major parasitic disease with a substantial economic impact on the poultry industry in Nigeria; it is a major limitation to the production and marketing of poultry[3].

One of the most economically significant illnesses in the poultry sector is avian *coccidiosis*, which is brought on by several species of parasitic apicomplexan protozoa of the genus *Eimeria*[4][5]. The parasitic illness that has the biggest financial impact on the global chicken business is *coccidiosis*[6][7]. The yearly costs of *coccidiosis* to the poultry industry worldwide are estimated to be over 14.4 billion US dollars, based on estimations of production losses and the financial impact of prophylactic *measures*[8].

Impaired feed conversion, decreased growth, downgrading at processing, death, and the expense of regular preventative and therapeutic medicines are the main causes of economic losses[9][10]. Although *coccidiosis* in chickens is typically caused by seven species of *Eimeria—E. tenella, E. necatrix, E.* acervulina, E. maxima, E. *brunetti,* E. praecox, and E. mitis—the first four pose the biggest threat to chicken production, primarily because of their combination of pathogenicity and distribution[11][12]. Clinical symptoms include diarrhea, dehydration, weight loss, rectal prolapse, dysentery, and frequently death result from extensive destruction to the intestinal *epithelia*[13]. Chickens may experience immunosuppression in a subclinical form, which can lead to secondary illness states, particularly necrotic enteritis brought on by Clostridium *perfringens*[14][15][16].

The three most common species in broiler chickens are *Eimeria tenella, E. acervulina, and E. maxima*[17][18]. *The most harmful parasite that parasitizes developing chicks is Eimeria tenella, which costs the poultry business a significant amount of money*[19][20]. Because of the species' presence in the caeca, caecal or "bloody" *coccidiosis* is characterized by inflammation and hemorrhage. Bloody droppings and the buildup of blood in the caecal lumen are indicators of it[21].

The two main methods of control are chemoprophylaxis, which is the mainstay in the control of *coccidiosis*[22][23][24], and, to a lesser extent, vaccination. However, the widespread and prolonged use and misuse of the drugs have led to the emergence of drug-resistant strains of *Eimeria* species. The cost of prophylactic administration of *anticoccidials* is high. In parallel, the development of new *anticoccidials* is prohibitively high, and coupled with the possible future bans on the use of *anticoccidial* drugs in commercial poultry production, there is an urgent need for novel approaches and alternative control strategies[25][26]. Vaccination as a means of control faces the cost of production as live vaccines are produced using living birds[27]. The attenuated forms of the vaccines can revert to virulence and cause clinical *coccidiosis.*

Recently, some trials have been conducted on the efficacy of some herbal products, and many have shown measurable efficacy in the prevention of *coccidiosis*[28][29][30]. Plants of the genus *Vernonia* are widely used in *ethnomedicine* and *ethnoveterinary* practice, as extensively reviewed [31][32][33]. There are documents on the *antiparasitic* efficacy of *V. amygdalina*[34][35][36][37]. In addition, reports also abound on the use of the plant extracts in the treatment of *apicomplexan Protozoa*[38][39][40][41].

But only by adding them to broiler feed, which reduces oocyst generation and increases bird weight gain, have the beneficial effects of V. *amygdalina* against avian coccidiosis been proven[42][43][44]. Therefore, a thorough investigation into V. *amygdalina's anticoccidial* properties in broiler chicks is required. It is hoped that the findings of this investigation would advance knowledge of *V. amygdalina's anticoccidial* properties. In order to assess the *anticoccidial* properties of *V. amygdalina* leaf *methanolic* extract and its main fractions, this study will be conducted on broiler chickens that have been experimentally infected with Eimeria tenella.

Materials and Methods

Plant collection, identification, and processing

At the flowering stage, a single batch of fresh, disease-free *V. amygdalina* leaves was gathered from a private garden in Jos town. Jos is situated between longitudes 8o 32' and 10o 38' E and latitudes 8o 24' N. It is made up of plains, hills, and depressions of different sizes, and its average elevation is 1,250 meters above sea level. October through April is the dry season, and May through September is the wet season. December and January temperatures are below 15°C, and there is an average of 1,100 mm of rainfall every year[45]. Voucher sample number 7183 was placed in the Department of Biological Sciences' herbarium for future use after the sample was identified and verified at Ahmadu Bello University in Zaria. After being cleaned with tap water, the leaves were allowed to air dry on galvanized wire screens in the shade, occasionally moving, until their weight remained consistent. Ten kilograms (10 kg) of leaves were then ground into a powder using a mechanical grinder and kept in an airtight plastic container until they were needed.

Extraction and fractionation of *V. amygdalina* leaves

In a Soxhlet system, a 2 kg sample of the powdered leaves of V. *amygdalina* were extracted using 10 L of absolute methanol at 70 oC[46]. in order to produce a *methanolic* extract. A rotary evaporator was used to turn the extract into powder at 40 oC, and it was then refrigerated until it was needed in an airtight amber-colored glass bottle[47].

Phytochemical Analysis

Using conventional protocols as outlined by *Hashemi et al.*, chemical tests were conducted to screen and identify the bioactive chemical elements of the plant's methanolic leaf extract[48].

Parasite propagation and purification

The study made use of a local strain of *E. tenella* that had previously undergone molecular characterisation and was kept at Ahmadu Bello University's Department of Veterinary Parasitology and Entomology in Zaria[49].

To propagate the oocysts, a seed stock of 1 x1 0^4oocysts suspended in 1 mL of distilled water was administered orally to 5

broiler chickens at two weeks of age as recommended by Holdsworth *et al*.[50]. Large numbers of sporulated oocysts were then obtained and used for the subsequent main experiment[51]. Sporulated oocysts were cleaned and counted by the McMaster technique[52]. The required concentration of sporulated oocysts (100,000/mL) was maintained with phosphate-buffered saline.

Experimental birds, housing, and management

Three hundred (300) heads of one-day-old broiler chicks were purchased, brooded and managed in an isolated fly-proof pen designed to prevent contamination of extraneous coccidial infection. The brooder pen had litter composed of wood shavings to a depth of 5 cm. To meet the nutrient requirement of the broilers during the experimental period, a coccidiostat-free commercial diet formulated for the two stages of growth and water were provided *ad libitum*, and birds were routinely vaccinated against Newcastle disease and *Gumboro* disease.

Experimental design

A randomized full block design was used to conduct the experiment. As advised by Holdsworth *et al.*, 210 broilers with comparable body weights were chosen for the investigation[50][44]. The birds were divided into seven experimental groups, A–G, each of which had two replicates of fifteen birds.

As directed, 100,000 *sporulated oocysts* were implanted into each chick on day 21 of life[50][53][54]. Group A, which acted as a negative control, was the exception. In accordance with the following schedule, birds received treatment from one day before infection to five days after infection (Table 1). Every treatment was given in accordance with OS.

Table 1: Experimental infection and treatment of Broiler chickens

Group	Infection status	Treatment	Duration
A - Negative control (NC)	Uninfected	Normal saline	*ad libitum*
B - Positive control (PC)	Infected	Normal saline	*ad libitum*
C - Standard control (SC)	Infected	Amprolium 125 mg/kg	7 consecutive days
D - Methanolic extract (ME)	Infected	ME 1,000 mg/kg	7 consecutive days
E - Hexane fraction (HF)	Infected	HF 500 mg/kg	7 consecutive days
F - Aqueous fraction (AF)	Infected	AF 500 mg/kg	7 consecutive days
G - Butanol fraction (BF)	Infected	BF 500 mg/kg	7 consecutive days

Parameters used to assess anticoccidial efficacy include clinical signs, mortality, faecal oocysts count, faecal score, lesion score, weight gain, feed intake, and feed conversion ratio according to standard procedures.

Ethical approval

Ethical approvals for the use of live animals were obtained from the Ethical Committee of the National Veterinary Research Institute, Vom, and that of Ahmadu Bello University, Zaria

Data analysis

The body weight gain, FCR, lesion score, and oocyst counts were expressed as the mean and pooled standard error (±SE). The mean values were compared by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Statistical analysis for categorical lesion score and faecal score data was assessed using the Kruskal-Wallis test and separated by Dunn's test. The statistical analysis was performed with SPSS version 20.0. The differences between groups were considered significant if $p<0.05$.

Results

Phytochemical composition

The results of the phytochemical analysis of methanolic extracts of *Vernonia amygdalina* leaf have been reported in a previous work[55].

Clinical Findings

Classical signs of caecal coccidiosis, including depressed feed and water intake, ruffled feathers, wing drooping, huddling, and haemorrhagic diarrhea, were observed in all infected groups at d 5 pi. In addition, prostration and somnolence were observed in some of the birds. Reduction in the severity of the clinical signs was conspicuously observed in birds of groups D-G.

Mortality rate

Mortality of chickens in the magnitude of 13.33% was recorded for birds in the uninfected unmedicated group, as presented in Table 2. In terms of mortality, chickens treated with methanol extract, the hexane fraction, aqueous residue fraction, and butanol fraction were all devoid of death.

Fecal score of birds on days 5, 6, and 7 post-infection

On day 5, the faecal score was highest in the PC group (3.00) and lowest in the BF group (1.00). In the same manner, the faecal score was highest in the PC (2.50) group and lowest in the ME, HF, and BF with scores of 1.50, 2.00, and 1.00 on day 6 pi, respectively. The faeces of infected birds all returned to the normal values except for the PC group, which had an average faecal score of 1.50 by d 7 pi (Table 3).

Lesion Score

The caeca lesions of birds selected from each group was determined 6 d pi (Table 4). The lesion score of 3.00 was significantly (<0.05) higher in group PC group compared to groups treated with the methanolic extract, and hexane, aqueous, and butanol fractions which had scores of 1.00, 1.50, 2.00, and 1.00, respectively.

Oocyst output

The oocysts produced and excreted by experimental birds on 5, 6, 7 and 14 dpi is presented in Table 5. The highest number of oocysts (125, 212 opg) was shed by birds in the positive control group on d 7 pi, whereas the lowest among the treated groups were shed by chickens in the butanol fraction group (762). The birds in

all the groups did not shed further oocysts by 14 dpi except those in the positive control group which passed few (325) opg.

Performance of birds

The performance of birds as assessed by body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) is presented in Table 6. Significantly ($p < 0.05$) higher body weight gain was recorded in the NC treatment and significantly ($p < 0.05$) lower body weight gain was observed in the PC treatment in the first week pi but, there were no significant ($p > 0.05$) difference in mean body weight gain among the various treatments 2 weeks pi.

As shown in Table 6, there were no significant differences in feed intake among the groups in weeks 1 and 2 pi. The feed conversion ratio (FCR) was significantly ($p < 0.05$) higher in the PC treatment in the first week pi than in birds of the other groups.

Table 2: Mortality rate in birds experimentally infected with E. tenella and treated with the methanolic leaf extract of V. amygdalina and its fractions.

Group	Treatment	% Mortality
A	Negative control	0.00[a]
B	Positive control	13.33[b]
C	Standard control	0.00[a]
D	Methanolic extract	0.00[a]
E	Hexane fraction	0.00[a]
F	Aqueous fraction	0.00[a]
G	Butanol fraction	0.00[a]

a, b differ significantly ($p<0.05$) from one another

Table 3: Faecal scores of birds infected with *E. tenella* and with methanolic leaf extract of *V. amygdalina* and its fractions determined on 5, 6 and 7 dpi.

Group	Treatment	Days Post Infection		
		5	6	7
A	Negative control	0.00[c]	0.00[b]	0.00[b]
B	Positive control	3.00[a]	2.50[a]	1.50[a]
C	Standard control	0.00[c]	0.00[b]	0.00[b]
D	Methanol extract	1.50[b]	1.00[b]	0.00[b]
E	Hexane fraction	2.00[ab]	1.00[b]	0.00[b]
F	Aqueous fraction	2.00[ab]	1.00[b]	0.00[b]
G	Butanol fraction	1.00[bc]	1.00[b]	0.00[b]
	SE±	0.189	0.189	0.268

a, b, c differ significantly ($p<0.05$) from one another

Table 4: Lesion score of the caeca of birds infected with E. tenella and treated with methanolic leaf extract of V. amygdalina and its fractions determined at 6 dpi.

Group	Treatment	Lesion Score
A	Negative control	0.00[c]
B	Positive control	3.50[a]
C	Standard control	0.00[c]
D	Methanol extract	1.00[bc]
E	Hexane fraction	1.50[b]
F	Aqueous fraction	2.00[b]
G	Butanol fraction	1.00[bc]
	SE±	0.268

a, b, c differ significantly ($p<0.05$) from one another

Table 5: Oocyst output of birds experimentally infected with *E. tenella* and treated with the methanolic leaf extract of *V. amygdalina* and its fractions at 5, 6, 7 and 14 days post infection.

		Oocyst Output			
		Days Post Infection			
Group	**Treatment**	**5**	**6**	**7**	**14**
A	Negative control	0.00[d]	0.00[c]	0.00[c]	0.00[b]
B	Positive control	2,375.00[a]	47,662.50[a]	125,212.50[a]	325.00[a]
C	Standard control	0.00[d]	0.00[c]	0.00[c]	0.00[b]
D	Methanolic extract	237.50[c]	1,150.00[c]	612.50[c]	0.00[b]
E	Hexane fraction	425.00[b]	1,387.50[c]	1,487.50[c]	0.00[b]
F	Aqueous fraction	237.50[c]	5,037.50b	6,325.50[b]	0.00[b]
G	Butanol fraction	0.00[d]	187.50[c]	762.50[c]	0.00[b]
	SE±	31.576	438.850	520.645	13.082

a, b, c differ significantly (p<0.05) from one another

Table 6: The effects of treatments on body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) of broiler chickens at the end of the first and second weeks pi with *E. tenella.*

		Performance Post Infection					
		Weeks Post Infection					
		Week 1			Week 2		
Groups	**Treatment**	**BWG (g)**	**FI (g)**	**FCR**	**BWG (g)**	**FI (g)**	**FCR**
A	Negative control	294.65[a]	590.60	2.01[b]	384.28	1010.39	2.63
B	Positive control	182.59[c]	582.43	3.20[a]	321.92	1121.32	3.50
C	Standard control	257.34[ab]	637.95	2.48[ab]	378.08	1007.16	2.67
D	Methanol extract	245.90[b]	588.84	2.40[b]	371.15	1001.35	2.71
E	Hexane fraction	241.41[b]	585.74	2.31[b]	348.43	997.89	2.88
F	Aqueous fraction	243.08[b]	585.00	2.41[b]	330.77	999.96	3.03
G	Butanol fraction	276.80[ab]	590.14	2.14[b]	396.01	1005.73	2.54
	SE±	8.052	16.078	0.130	15.909	35.935	0.201

a, b, c differ significantly (p<0.05) from one another

Discussion

Typical signs of coccidiosis, particularly haemorrhagic diarrhoea, depressed feed intake, ruffled feathers, huddling and depression were observed in broiler chickens in the positive control group. However, the signs were absent or milder in the groups treated with the methanolic extract of *V. amygdalina* leaf and its fractions. The finding in this study concurs with the haemorrhagic diarrhea, reduced feed intake, ruffled feathers, huddling, and depression observed in similar studies[56][57][58][59]. The progressive reduction in the severity of the observed clinical signs in the treated groups compared with the infected but untreated group could be attributed to the anticoccidial effects of the phytoconstituents in the leaf of the plant. *V. amygdalina* leaf administered in the feed of broiler chickens has been shown to inhibit the invasion of caecal endothelial cells by sporozoites; this supports its anticoccidial role as *Eimeria* parasites must invade, and multiply in the endothelial cells of the caecum before infection is established[60][61]. Outbreak of coccidiosis in a poultry farm usually results in a devastating economic loss as a result of the high morbidity and variable mortality associated with the disease[62]. The mortality of 13% was only recorded for broiler chickens in the positive control group in this study. The methanolic extract of *V. amygdalina* leaf and its fractions likely contained phytochemicals that ameliorated the debilitating effect of coccidiosis in the treated birds and eliminated the mortality that is usually associated with the disease.

The bloody diarrhoea observed in all the infected groups except in the standard treatment group, from the fifth to the seventh day post infection is in agreement with the findings of other authors[63][64]. The intensity of the bloody diarrhoea was significantly lower in the treated broiler chickens compared with those in the positive control group. In similar studies using other plant extracts[65][66], bloody diarrhoea was observed in infected chickens compared with the uninfected control. The limited invasion of the epithelial cells of the caeca of infected birds as shown by scanty pathological lesions might have resulted in the limited production of schizonts and subsequently fewer gametocytes with resultant decreased disruption of blood vessels and reduced blood in the faeces.

This study also used gross lesion score as an indicator of the anticoccidial effect of the methanolic extract of *V. amygdalina* leaf and its fractions. Macroscopic lesions were significantly less severe in the group of birds infected and treated with the methanolic extract of *V. amygdalina* leaf and its fractions compared with birds in the positive control group. This suggests that the methanolic extract of *V. amygdalina* and fractions contain substances that protect the caecum from excessive damage by *E. tenella.*

The number of oocysts excreted in the faeces of birds may be an important indicator of the severity of coccidiosis[67][68]. In the present study broiler chickens in the groups infected and treated with methanolic extract of *V. amygdalina* leaf and its fractions excreted significantly fewer oocysts than birds in the positive control group. This suggests that the methanolic extract of *V. amygdalina* leaf and its fractions had an ameliorative effect on oocysts excretion and may play significant role in the control of avian coccidiosis. Several plant extracts and their derived phytochemicals have displayed efficacy in the control of coccidiosis in experimental studies adjudged by their ability to reduce oocyst output in infected chickens[69][70][71]. Performance, evaluated in the forms of feed intake, weight gain, and feed

conversion ratio, has been recognised and extensively measured in the evaluation of the efficacy of anticoccidial products[72][73][74][75]. The degree of protection against coccidiosis defined by the body weight gain is an acceptable and reliable measure for quantifying the efficacy of control by anticoccidials[76]. The efficacy of plant extracts and phytoconstituents has been determined on comparative body weight gain following experimental challenge with coccidiosis as described previously[77][53][54].

In this study, weight gain was broadly similar for all the treated groups compared to that recorded in the negative control group. A significant effect of treatments with methanolic extract of *V. amygdalina* leaf and its major fractions was noticeable, as the positive control group showed significantly lesser body weight gain compared with all treatments. The reduction in the body weight gain in the positive control group was not surprising because coccidial infections are known to cause extensive damage to the intestinal mucosa with resultant nutrients malabsorption and subsequent reduction in weight gain[67][78]. In addition, parasitic infection is known to result in nutrient resource allocation to immune response, which result is evident difference in body weight gain. Numerous studies investigating the effects of dietary inclusion of plant extracts have revealed improved weight gain in experimentally induced coccidial infections[57][79][30]. Variations in weight gain may occur due to reasons advanced by Crouch *et al*.[80], who maintained that some breeds of broiler chickens, when raised in cages were more varied in their weight gain to virulent coccidiosis challenge.

In this study, there were less remarkable differences in feed intake among birds in the different treatment groups compared with the birds in the positive control group. The anticoccidial efficacy of the methanolic extract of *V. amygdalina* leaf and its major fractions as adjudged by feed intake indicated effective control against infection with *E. tenella.* Other authors recorded similar results to this finding, where several experiments were conducted to study the effects of herb extracts on performance, particularly, feed intake during coccidiosis[81][82]. In contrast, Nghonjuyi *et al*.[83] showed there was no significant difference in the feed intake between groups of Kabir chickens infected with *Eimeria* species and treated with leaf extract of *Carica papaya*. A major challenge encountered in this study was the accurate determination of feed intake. Waste of feed as a result of overturning of feeders was common place and the feed wasted could not be adequately measured.

Feed conversion ratio has also been used as a performance attribute to determine the effects of plant extracts against coccidiosis[84][70][85]. In this study, the birds in the positive control group exhibited the least value of feed conversion ratio. Inefficient feed conversion ratio as a result of experimentally-induced coccidiosis has been reported by Kurkure *et al*.[86] who recorded lower feed conversion ratio value for coccidiosis-infected broiler chickens medicated with a proprietary polyherbal preparation "coxynil" as compared to positive control birds. Similar findings are also documented by Kheirabadi *et al*.[87] who stated that birds treated with granulated extracts of *Artemisia sieberi* had better feed conversion ratio when infected with *Eimeria* parasites. These findings are strongly supported on the research of Tanweer *et al*.[57] who observed a lower feed conversion ratio value for broiler chickens infected with coccidial parasites and treated with *Peganum harmala* extracts.

Conclusion

This study has demonstration that methanolic leaf extracts of *Vernonia amygdalina* and its major fractions have anticoccidial activity against *Eimeria tenella* in broiler chickens. Methanolic extract at 1,000 mg/kg body weight showed profound activity on the parameters measured compared to the other fractions. It is therefore, recommended that thorough phytochemical analysis be conducted on the leaf of *V. amygdalina* to identify the phytocomponent(s) that may be responsible for these biological activities and as well, determine their mode(s) of action.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgement

The authors acknowledge the Head, Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, Nigeria, and the staff of the Protozoology Unit of the Department, for the assistance rendered during the laboratory phase of this work. The staff of the Parasitology Division at the National Veterinary Research Institute Vom, Plateau State, Nigeria, are also highly appreciated for the assistance rendered in the course of the research.

References

1. Nnadi, P. A. and George, S. O. (2010). A cross-sectional survey on parasites of chickens in selected villages in sub-humid zones of South-Eastern Nigeria. *Journal of Parasitology Research*, Article ID141824, 6 pages.
2. Akintunde, O. K. and Adeoti, A. I. (2014). Assessment of factors affecting the level of poultry disease management in southwest, Nigeria. *Trends in Agricultural Economics*, 7(2): 41-56.
3. Musa, I. W., Sa'idu, L., Jatau, I. D., Adamu, J., Otu, M.O. and Abdu, P. A. (2010). Outbreak of coccidiosis in 5-day old commercial breeder flock in Zaria, Nigeria. *International Journal of Poultry Science*, 9(12): 1112-1115.
4. Allen, P. C. and Fetterer, R. H. (2002). Recent advances in biology and immunobiology of *Eimeria* species and in diagnosis and control of infection with these coccidian parasites of poultry. *Clinical Microbiology Reviews*, 15(1): 58–65.
5. Chapman, H. D., Barta, J. R., Blake, D., Gruber, A., Jenkins, M., Smith, N. C., Suo, X. and Tomley, F. M. (2013). A selective review of advances in coccidiosis research. *Advances in Parasitology*, 83:93-171.
6. Chapman, H. D., Jeffers, T. K. and Williams, R. B. (2010). Forty years of monensin for the control of coccidiosis in poultry. *Poultry Science*, 89: 1788-1801.
7. Chapman, H. D. (2014). Milestones in avian coccidiosis research: a review. *Poultry Science*, 93:501-511.
8. Blake, D., Knox, J., Dehaeck, B., Huntington, B., Rathinam, T., Ravipati, V., Ayoade, S., Gilbert, W., Adedambo, A., Jatau, I., Raman, M., Parkar, D., Rushton, J. and Tomley, F. (2020). Re-calculating the cost of coccidiosis in chickens. *Veterinary Research*, 51: 115.
9. Williams, R. B. (1999). A compartmentalized model for the estimation of the cost of coccidiosis to the world's chicken production industry.*International Journal for Parasitology*, 29: 1209 – 1229.

10. Girgis, G. (2007). Coccidiosis: a field problem with many aspects to consider. *World Poultry*, 8: 44-45.
11. Chapman, H., Cherry, T., Danforth, H., Richards, G., Shirley, M. and Williams, R. (2002). Sustainable coccidiosis control in poultry production: the role of live vaccines. *International Journal for Parasitology*, 32: 617-629.
12. Shah, A. A. A., Song, X., Xu, L., Yan, R., Song, H., Ruirui, Z., Chengyu, L. and Li, X. (2009).The DNA-induced protective immunity with chicken interferon gamma against poultry coccidiosis. *Parasitology Research*, 107: 747 – 750.
13. Cox, F. E. (1998). Control of coccidiosis: lesson from other protozoa. *International Journal for Parasitology*, 28: 617-629.
14. Sivaseelan, S., Viyakum, S., Malmarugam, S., Balachandran, P. and Balasubramaniam, G. A. (2013). Assessment of predisposing effect of coccidiosis to necrotic enteritis in experimentally induced broiler chicken. *Veterinarski Arhiv*, 83(6): 653-664.
15. Chan, G., Guthrie, A., Sivaramalingam, T., Vancraeynest, D., Moody, R. and Clark, S. (2016). A framework for assessing the efficacy of antimicrobials in the control of necrotic enteritis in broiler chickens. *Journal of Applied Poultry Research*, 25(2): 246-256.
16. Moore, R. J. (2016). Necrotic enteritis predisposing factors in broiler chickens. *Avian Pathology*, 45: 275-281.
17. Gyorke, A., Pop, L. and Cozma, V. (2013). Prevalence and distribution of *Eimeria* species in broiler chickens farms of different capacities. *Parasite*, 20: 50.
18. Chapman, H. D., Barta, J. R., Hafeez, M. A., Matsler, P., Rathinam, T. and Raccoursier, M. (2016). The epizootiology of *Eimeria* infection in commercial broiler chickens where anticoccidial drug programs were employed in six successive flocks to control coccidiosis. *Poultry Science*, 95:1774-1778.
19. Chapman, H. D. and Shirley, M. W. (2003). The Houghton strain of *Eimeria tenella*: a review of the type strain selected for genome sequencing. *Avian Pathology*, 32: 115-127.
20. Shirley, M. W., Smith, A. L. and Blake, D. P. (2007). Challenges in the successful control of avian coccidia. *Vaccine*, 25(30): 5540 – 5547.
21. McDougald, L. and Fitz-Coy, S. (2013). Coccidiosis: protozoan infections. In: Swayne, D.E (ed.). *Diseases of Poultry*, 13th edition. Blackwell, New York, pp. 1148-1166.
22. Peek, H. W. and Landman, W. J. M. (2011). Coccidiosis in poultry: anticoccidial products, vaccines and other prevention strategies. *Veterinary Quarterly*, 31: 143-161.
23. Tewari, A. K. and Maharana, B. R. (2011). Control of avian coccidiosis: changing trends. *Journal of Parasitic Diseases*, 35(1): 10-17.
24. Witcombe, D. M. and Smith, N. C. (2014). Strategies for anticoccidial prophylaxis. *Parasitology*, 141:1379-1389.
25. Giannenas, I., Papadopoulos, E., Tsalie, E., Triantafillou, E., Henikl, S., Teichmann, K. and Tontis, D. (2012). Assessment of dietary supplementation with probiotics on performance, intestinal morphology and microflora of chickens infected with *Eimeria tenella*. *Veterinary Parasitology*, 188: 31-40.
26. Yang, W. C., Tien, Y. J., Chung, C. Y., Chen, Y. C., Chiou, W. H., Liu, H. Y., Liang, C. L. and Chang, C. L. T. (2015). Effect of *Bidens pilosa* on infection and drug resistance of *Eimeria* in chickens. *Research in Veterinary Science*, 98:74-81.
27. Shirley M. W., Smith, A. L. and Tomley, F. M. (2005).The biology of avian *Eimeria* with an emphasis on their control by vaccination. *Advances in Parasitology*, 60: 285–330.
28. Habibi, H., Firouzi, S., Nili, H., Razavi, M., Asadi, S.L. and Daneshi, S. (2016). Anticoccidial effects of herbal extracts on *Eimeria tenella* infection in broiler chickens: *in vitro* and *in vivo* study. *Journal of Parasitic Diseases*, 40(2):401-407.
29. Gadelhaq, S. M., Arafa, W. M. and Abolhadid, S. M. (2018). *In vitro* activity of natural and chemical products on sporulation of *Eimeria* species oocysts of chickens. *Veterinary Parasitology*, 251:12-16.
30. Wang, D., Zhou, L., Li, W., Zhou, H. and Hou, G. (2018). Anticoccidial effects of areca (*Areca catechu* L.) extract on broiler chicks experimentally infected with *Eimeria tenella*. *Experimental Parasitology*. 184: 26-21.
31. Yeap, S.K., Ho, W.Y., Beh, B. K., Liang, W.S., Ky, H., Yousr, A.H.N. and Alitheen, N. B. (2010). *Vernonia amygdalina*, an ethnoveterinary and ethnomedical used green vegetable with multiple bioactivities. *Journal of Medicinal Plants Research*, 4(25): 2787-2812.
32. Toyang N. J and Verpoorte, R. (2013). A review of the medicinal potentials of plants of genus *Vernonia* (Asteraceae). *Journal of Ethnopharmacology*, 146:681-723.
33. Oyeyemi, I. T., Akinlabi, A. A., Adewumi, A., Aleshinloye, A. O. and Oyeyemi, O. T. (2018). *Vernonia amygdalina*: a folkloric herb with anthelminthic properties. *Beni-Suef University Journal of Basic and Applied Sciences*, 7(1):43-49.
34. Molgaard, P., Nielsen, S. B., Rasmussen, D. E., Drummond, R. B., Makaza, N. and Andreasen, J. (2001). Anthemintic screening of Zimbabwean plants traditionally used against schistosomiasis. *Journal of Ethnopharmacology*, 74: 257-264.
35. Innocent, T. and Deogracious, O. (2006).The anthelmintic activity of selected indigenous medicinal plants used by the Bayankole of Western Uganda. *Journal of Animal and Veterinary Advances* 5:712-717.
36. Adedapo, A. A., Otesile. A. T. and Soetan, K.O. (2007). Assessment of the anthelminthic efficiency of aqueous crude extract of *Vernonia amygdalina. Pharmaceutical Biology*, 45: 564-568.
37. Ademola I. O. and Eloff, J. N. (2011). Anthelminthic activity of acetone extract and fractions of *Vernonia amygdalina* against *Haemonchus contortus* eggs and larvae. *Tropical Animal Health and Production*, 43: 521-527.
38. Masaba, S. S. (2000). The antimalarial activity of *Vernonia amygdalina* Del (Compositae) *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 94: 694-695.

39. Omoregie, E. S., Pal, A., Darokar, M. P., Chanda, D. and Sisodia, B. (2010).*In vitro* and *in vivo* antiplasmodial activity and cytotoxicity of extracts from *Vernonia amygdalina* leaves. *Malaria Journal*, 9: 30.
40. Adiukwu, P. C., Amon A. and Nambatya, G. (2011). Pharmacognostic, antiplasmodial and antipyretic evaluation of aqueous extract of *Vernonia amygdalina* leaf. *International Journal of Biological and Chemical Sciences*, 5: 709-716.
41. Alawa, J. N., Carter, K. C., Nok., A. J., Kwanashie, H. O., Adebisi, S. S., Alawa, C. B. I. and Clements, C. J. (2012). Infectivity of macrophages and the histopathology of cutaneous lesions, liver and spleen is attenuated by leaf extract of *Vernonia amygdalina* in *Leishmania major* infected BALB/c mice. *Journal of Complement Integrative Medicine*, 9: 1-20.
42. Mohammed, A. A. and Zakariya'u, A.S. (2012). Bitter leaf (*Vernonia amygdalina*) as a feed additive in broiler diets. *Research Journal of Animal Sciences*, 6(3): 38-41.
43. Oyagbemi, T. O. and Adejinmi, J. O. (2012). Supplementation of broiler feed with leaves of *Vernonia amygdalina* and *Azadirachta indica* protected birds naturally infected with *Eimeria sp. African Journal of Biotechnology*, 11(33): 8407-8413. Available online at http://www.academicjournals.org/AJB DOI: 10.5897/AJB11.3190 ISSN 1684–5315
44. Osho, I.B., Akindahunsi, A., Igbasan, F.A. and Adekunle, D. J. (2014). Effect of orally administered bitter leaf (*Vernonia amygdalina*) extract on the growth performance and haematological parameters of broiler chickens. *Journal of Veterinary Medicine and Animal Health*, 6(10): 251-256.
45. Plateau Agricultural Development Programme (2002). *Annual Report*, 2002.
46. Youn, H. J., Lakritz, J., Kim, D.Y., Rothingars, G. E. and Marsh, A. E. (2003). Antiprotozoan efficacy of medicinal herbs extracts against *Toxoplasma gondii* and *Neospora caninum*. *Veterinary Parasitology*, 116: 7-14.
47. Momoh, M., Adiukwu, M. and Oyi, A. R. (2010). *Vernonia amygdalina* and CD4+ cell counts: an immune study. *Global Journal of Biotechnology and Biochemistry*, 5: 92-96.
48. Hashemi, S.R, Zulkifli, I., Hair Bejo, A., Farida, A.and Somchit, M. N. (2008). Acute toxicity and phytochemical screening of selected herbal aqueous extract in broiler chickens. *International Journal of Pharmacology*, 4(5):352-360.
49. Jatau, I. D., Lawal, I. A., Kwaga, J. K. P., Tomley, F. M., Blake, D. P. and Nok, A. J. (2016). Three operational taxonomic units of *Eimeria* are common in Nigerian chickens and may undermine effective molecular diagnosis of coccidiosis. *BMC Veterinary Research*, 12: 86.
50. Holdsworth, P. A., Conway, D. P., McKenzie, M. E., Dayton, A. D., Chapman, H. D., Mathis, G. R., Skinner, J., Mudt, H C., and Williams, R. B. (2004). World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of anticoccidial drug in chickens and turkeys. *Veterinary Parasitology*, 121: 189-212.
51. Abu-Akkada, S. S. and Awad, A. M. (2015). Protective Effects of Probiotics and Prebiotics on *Eimeria tenella*-Infected Broiler Chickens. *Pakistan Veterinary Journal*, 35(4): 446-450. Accessible at: www.pvj.com.pk
52. Shirley, M. W. (1995). *Eimeria* species and strains of chickens. In:Eckert. J, Braun, R. Shirley, M.W. Coudert, P. (Eds.) *Biotechnology–Guidelines on Techniques in Coccidiosis Research*. Luxemburg: European Commission, Pp.1-24.
53. Meskerem, A. and Boonkaewwan, C. (2013). Protective effects of *Moringa stenopetala* leaf supplemented diets on *Eimeria tenella* infected broilers chickens in Debre Zeit, Central Ethiopia. *Kasetsart Journal (Natural Science)*, 47:398-406
54. Meskerem, A. and Boonkaewwan, C. (2014). Effect of *Lepidium sativum* L. (Garden Cress) seed and its extract on expereimental *Eimeria tenella* infection in broiler chickens. *Kasetsart Journal (Natural Science)*, 48: 28-37.
55. Biallah, M. B., Lawal, I. A., Abdu, P. A., Okubanjo, O. O. and Kaze, P. D. (2022). Phytochemical Profile of the Methanolic Leaf Extract of *Vernonia amygdalina* Del. and *In Vitro* Anticoccidial Effect of Its Fractions against *Eimeria tenella. International Journal of Innovative Research and Development*, 11(11): 67-72.
56. Jordan, A., Caldwell, D. J., Klein, J., Coppedge, J., Pow, S., Fitz-Coy, S. and Lee, J. T. (2011). *Eimeria tenella* oocysts shedding and output in caecal and faecal contents following experimental challenge in broilers. *Poultry Science*, 90: 990-995.
57. Tanweer, J. A., Chand, N., Saddique, U., Bailey, C.A. and Khan, R.U. (2014). Antiparasitic effect of wild rue (*Peganum harmala* L.) against experimentally induced coccidiosis in broiler chicks. *Parasitology Research*, 113(8): 2951-2960.
58. Lan, L., Zuo, B. Ding, H., Huang, Y., Chen, X. and Du, A. (2016). Anticoccidial evaluation of a traditional Chinese medicine-*Brucea javanica*-in broilers. *Poultry Science*, 95:811-818.
59. Jiao, J., Yang, Y., Liu, M., Li, J., Cui, Y., Yin, S. and Tao, J. (2018). Artemisinin and *Artemisia annua* leaves alleviate *Eimeria tenella* infection by facilitating apoptosis of host cells and suppress inflammatory response. *Veterinary Parasitology*, 254: 271-177.
60. Al-Fifi, Z. I. A. (2007). Effect of leaves extract of *Carica papaya*, *Vernonia amygdalina* and *Azadirachta indica* on the coccidiosis of free-range chickens. *Asian Journal of Animal Sciences*, 1(1): 26-32.
61. Muthamilselvan, T., Kuo, T. F., Wu, Y. C. and Yang, W. C. (2016). Herbal remedies for coccidiosis control: a review of plants, compounds and anticoccidial actions. *Evidence-Based Complementary and Alternative Medicine*. Volume 2016, Article ID: 2657981, 19 pages.
62. Quiroz-Castaneda, R. E. and Dantan-Gonzalez, E. (2015). Control of avian coccidiosis: future and present natural alternatives. *BioMed Research International*, Volume 2015, Article 430610, 11 pages.
63. Kostadinovic, L., Levic, J., Galonja-Coghill, T. and Ruzicic, L. (2012). Anticoccidian effects of *Artemisia absinthium* L. extracts in broiler chickens. *Archiva Zootechnica*, 15(2): 69-77.

64. Jenkins, M. C., Parker, C. and Ritter, D. (2017). *Eimeria* oocysts concentrations and species composition in litter from commercial broiler farms during anticoccidial drug or live *Eimeria* oocyst vaccine control programs. *Avian Diseases*, 61(2): 214-220.
65. Zaman, M. A., Iqbal, Z., Abbas R. Z. and Khan. M. N (2012). Anticoccidial activity of herbal complex in broiler chickens challenged with *Eimeria tenella*. *Parasitology*, 139: 237-243.
66. Abba, A. M. and Udo, E. J. (2018). *In-vivo* evaluation of anti-coccidial efficacy of aqueous extract of *Allium sativum* and *Carica papaya* in experimentally infected broiler chicks. *Open Access Journal of Biomedical Engineering and Biosciences*, 2(5)-2018, 9 pages.
67. Lee, J. J., Kim, D. H., Lim, J. J., Kim, D. G., Min, W., Kim, G. S., Lee, H. J., Rhee, M. H., Park, H., Kim, S. C., Chang, H. H. and Kim, S. (2012). Anticoccidial effect of supplemental dietary *Galla rhois* against infection with *Eimeria tenella* in chickens. *Avian Pathology*, 41(4): 403-407.
68. You, M. J. (2014).The comparative analysis of infection pattern and oocyst output in *Eimeria tenella*, *E. maxima* and *E. acervulina* in young broiler chicken. *Veterinary World*, 7(7):542-547.
69. Hong, S., Oh, G.W., Kang, W.G. and Kim, O. (2016). Anticoccidial effects of the *Plantago asiatica* extract on experimental *Eimeria tenella* infection. *Laboratory Animal Research*, 32(1):65-69.
70. Abbas, A., Iqbal, Z., Abbas, R. Z., Khan, M. K., Khan, J. A., Hussain, K., Mahmood, M. S. and Rizwan, H. M. (2017). Immunomodulatory effects of *Camellia sinensis* against coccidiosis in chickens. *Journal of Animal and Plant Sciences*, 27(2): 415-421.
71. Ahad, S., Tanveer, S., Nanchoo, I.A. and Malik, T.A. (2017). Anticoccidial activity of *Artemisia vestita* (Anthemideae, Asteraceae), a traditional herb growing in Western Himalayas, Kashmir, India. *Microbial Pathogenesis*, 104: 289-295.
72. Sun, H., Wang, L., Wang, T., Zhang, J., Liu, Q., Chen, P., Chen, Z., Wang, F., Li, H., Xiao, Y. and Zhao, X. (2014). Display of *Eimeria tenella* EtMic2 protein on the surface of *Saccharomyces cerevisiae* as a potential oral vaccine against chicken coccidiosis. *Vaccine*, 32: 1869-1876.
73. Ritzi, M. M., Abdelrahman, W., Monl, M. and Dalloul, R. A. (2014). Effects of probiotics and application methods on performance and response of broiler chickens to an *Eimeria* challenge. *Poultry Science*, 93(11): 2772-2778.
74. Ahad, S., Tanveer, S. and Malik, T.A. (2016). Anticoccidial activity of aqueous extract of a wild mushroom (*Ganoderma applanatum*) during experimentally induced coccidial infection in broiler chicken. *Journal of Parasitic Diseases*, 40(2): 408-414.
75. Hessenberger, S., Schatzmyar, G. and Teichman, K. (2016). *In vitro* inhibition of *Eimeria tenella* sporozoites invasion into host cells by probiotics. *Veterinary Parasitology*, 229:93-98.
76. Albanese, G. A., Tensa, L. R., Aston, E. J., Hilt, D. A. and Jordan, B. J. (2018). Evaluation of a coccidia vaccine using spray and gel applications. *Poultry Science*, 97(5): 1544-1553.
77. Jiang, L., Lin, J., Han, H., Dorg, H., Zhao, Q., Zhu, S. and Huang, B. (2012). Identification and characterization of *Eimeria tenella* apical membrane antigen-1 (AMA 1). *PLoS One*, 7(7): e41115.
78. Awais, M. M., Akhtar, M., Anwar, M. I. and Khaliq, K. (2018). Evaluation of *Saccharum officinarum* L. bagasse-derived polysaccharides as native immunomodulatory and anticoccidial agents in broilers. *Veterinary Parasitology*, 249:74-81.
79. Chauhan,S., Singh, V. S. and Thakur, V. (2017). Effect of *Calotropis procera* (madar) and amprolium supplementation on parasitological parameters of broilers during mixed *Eimeria* species infection. *Veterinary World*, 10(8): 864-868.
80. Crouch, C. F., Andrews, S. J., Ward, R. G. and Francis, M. J. (2003). Protective efficacy of a live attenuated anticoccidial vaccine administered to 1-day-old chickens. *Avian Pathology*, 32(3): 297-304.
81. Wang, D., Zhou, H., Li, W., Zhou, H. and Hou, G. (2016). Anticoccidial effect of *Piper sarmentosum* extracts in experimental coccidiosis in broiler chickens. *Tropical Animal Health and Production*, 48(5):1071-1078.
82. Ullah, M.I., Akhtar, M., Awais, M.M., Anwar, M. I. and Khaliq, K. (2018). Evaluation of immunostimulatory and immunotherapeutic effects of tropical mushroom (*Lentinus edodes*) against eimeriasis in chicken. *Tropical and Animal Health and Production*, 50:97-104.
83. Nghonjuyi, N. W., Tiambo, C. K., Kimbi, H. K., Manka'a, C. N., Juliano, R. S. and Lisita, F. (2015a). Efficacy of ethanolic extract of *Carica papaya* leaves as a substitute of sulphanomide for the control of coccidiosis in KABIR chickens in Cameroon. *Journal of Animal Health and Production*, 3(1): 21-27.
84. Malik, T. A., Kamili, A. N., Chishti, M. Z., Tanveer, S., Ahad, S. and Johri, R. K., (2014). *In vivo* anticoccidial activity of berberine [18,5,6-dihdro-9,10-dimethoxybenzo(g)-1,3-benzodioxolo(5,6-a) quinolizinium] – an isoquinoline alkaloid present in the root bark of *Berberis lycium*. *Phytomedicine*, 21(5):663-669.
85. Fatemi, A., Asasi, K. and Razavi, S. M. (2017). Anticoccidial effects of *Artemisia annua* ethanolic extract: prevention, simultaneous challenge-medication, and treatment. *Parasitology Research*, 116(9): 2581-258.
86. Kurkure, N. V., Kolte, S. W., Bhandakrar, A. G. and Kalorey, D.R. (2006).Evaluation of herbal coccidiostat "coxynil" in broiler. *Indian Journal of Experimental Biology*, 44:740-744.
87. Kheirabadi, K., Katadj, J., Bahadoran, S., Texeira da Silva, J. A., Samani, A. D. and Bashi, M. C. (2014). Comparison of the anticoccidial effect of granulated extract of *Artemisia sieberi* with monensin in experimental coccidiosis in broiler chickens. *Experimental Parasitology*, 141:129-133.