ANTICOCCIDIAL ACTIVITY OF AQUEOUS EXTRACT OF BITTER LEAF *(VERNONIA AMYGDALINA*) IN EXPERIMENTAL BROILER CHICKEN INFECTED WITH MIXED *EIMERIA* SPECIES.

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ABSTRACT
This study was carried out to determine the effect of aqueous extract of *Vernonia amygdalina* leaves in broiler chickens infected with mixed *Eimeria* species. The extract obtained was screened for phytochemicals, and the results revealed the presence of saponins, tannins, cardiac glycosides, alkaloids, flavonoids, steroids, and anthraquinones. The extract was tested on 20 experimentally infected broilers of 21 day-old, grouped into four (A, B C and D). The infection was carried out by giving a 2 ml solution containing 3000 oocysts of mixed *Eimeria* species to each broiler. Oocyst count was carried out for 3 days. There was no egg shedding within the first 2 days of infection, but significant egg shedding was observed on the third day. Group A was untreated, group B was treated with amprocox, while groups C and D were treated with the extract for 3 days post-infection. There was a 60% survival rate for the untreated group, while there was a 100% survival rate in the treated groups. Egg shedding was observed to be high 7 days post-treatment, with a significant difference between the groups. However, at 14 days post-treatment, egg shedding had reduced significantly, indicating the effectiveness of the extract, though there was no significant difference in egg shedding between the treated groups. Weight gain was observed across all treatment groups. From the results, the extract was able to reduce egg shedding, and no mortality was observed, indicating that it could be a good agent for the treatment of coccidiosis in broiler chickens.
Keywords
Coccidiosis, *Eimeria*, *Vernonia amygdalin*

INTRODUCTION

Coccidiosis is a disease caused by protozoan parasites belonging to *Apicomplexa* phylum, the family of the *Eimeridae* and genus of *Eimeria*. Seven species of *Eimeria* have been reported to be of pathological importance in chicken. These includes *E. acervulina, E. brunetti, E. maxima, E. necatrix, E. tenella, E. praecox* and *E. mitis* (Dakpogan *et al*., 2019). Coccidiosis remains a significant concern in commercial chicken production due to the high costs associated with its control. If left untreated, it can lead to reduced growth and high mortality rates (Dakpogan *et al*., 2019). As obligate intracellular parasites, *Eimeria* spp. infect epithelial cells (Kuraa *et al*., 2021). *E. intestinalis* infection damages intestinal epithelial cells and triggers an inflammatory response in the gut. Additionally, it induces a T helper 1 (Th1) immune response and increases levels of proinflammatory and anti-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), interleukin 12 (IL-12), IL-6, and IL-10 (Abdel-Haleem *et al*., 2017). The unregulated use of veterinary anticoccidials on farms has led to drug resistance, residues, and ecotoxicity. In the context of One Health, the increasing development of drug-resistant Coccidia species and veterinary drug residues has become a public health concern (Danquah *et al*., 2012), prompting the search for alternative treatments such as medicinal plants. Medicinal plants contain phytochemicals that are gaining attention as alternatives for treating coccidiosis due to their broad-spectrum biological activities and perceived safety.

Vernonia amygdalina, commonly known as bitter leaf, belongs to the *Asteraceae* family and is a rapidly regenerating shrub (Shui *et al.,* 2021*). Vernonia amygdalina* Del (Asteraceae) is a small shrub with dark green leaves and rough barks growing predominantly in tropical Africa but has been domesticated in many parts of West Africa. It is a perennial plant that grows to a height between 1-6 m (Nwosu *et al*., 2013).

The compound responsible for the bitter taste of bitter leaf is a sesquiterpene lactone known as vernodalin. The leaves are consumed as a green leafy vegetable and are reported to contain several minerals and vitamins (Sobukola *et al*., 2007). It has a wide range of use in folklore against diverse tropical diseases. More importantly, it has gained wide application in the treatment of amoebic dysentery (Moundipa *et al*., 2000), gastrointestinal disorders, antimicrobial and antiparasitic activities (Hladik *et al*., 2005). Some of the identified bioactive compounds in Vernonia amygdalina responsible for its ethnobotanical uses include alkaloids, saponins, terpenes, lignans, flavonoids, phenolic acids, steroids, anthraquinones, coumarins, sesquiterpenes, xanthones, and edotides (Muraina *et al*., 2010). Traditionally, *Vernonia amygdalina* has shown promising activities in this regard, and many scientific studies have been conducted and are currently ongoing to consolidate its acclaimed folklore antiparasitic use. There is also scientific evidence for the anthelmintic effect of *Vernonia amygdalina* in both humans and livestock. The raw leaf has been reported to affect the eggs of several nematodes of economic importance (Leonidas *et al*., 2013). More importantly, the aqueous extract of the plant has been reported to show greater efficacy against many livestock helminths than traditional treatments such as ivermectin, levamisole, and albendazole (Adediran and Uwalaka, 2015). Experimental studies have further shown the antischistosomal activities of sesquiterpene lactones and steroid glycosides from Vernonia amygdalina (Ogboli *et al*., 2000), as well as antiplasmodial (Abay *et al*., 2015) and anticancer activities (Owoeye *et al*., 2010).

There is also scientific evidence for the anti-helminthic effect of *Vernonia amygdalina* both in human and livestock. The raw leaf has been reported to have effect against the eggs of several nematodes of economic importance (Leonidas *et al.,* 2013). More importantly, the aqueous extract of the plant has been reported to show greater efficacy against many livestock helminths than traditional treatments such as ivermectin, levamisole, and albendazole (Adediran and Uwalaka, 2015). Experimental studies have further shown the antischistosomal activities of sesquiterpene lactones and steroid glycosides from Vernonia amygdalina (Ogboli *et al*., 2000), as well as antiplasmodial (Abay *et al*., 2015) and anticancer activities (Owoeye *et al*., 2010).

The aim of this study is to evaluate anticoccidial property of aqueous extract of *Vernonia amygdalina* leaves in broilers infected with mixed *Eimeria species* and the phytochemicals present.

MATERIALS AND METHOD

Study Design

The experiment was conducted using a completely randomized design, with 4 treatment groups, each consisting of 5 replications. A total of 20 broilers were subdivided into 4 groups (A, B, C, D), with 5 broilers in each group.

Sample collection and preparation

Vernonia amygdalina leaves were collected from the National Veterinary Research Institute, Vom. The leaves were washed, air-dried in the laboratory, pulverized using a wooden mortar and pestle, and stored in airtight jars for later use. *Vernonia amygdalina* leaves were collected from National Veterinary Research Institute Vom. The leaves were washed and air dried in the laboratory. The dried leaves were pulverised with a wooden mortar and pestle and stored in airtight jars for later use.

Preparation of the aqueous extract

The maceration method was used to extract 200 g of pulverized leaves in 500 ml of water and allowed to stand for 48 hours. The mixture was filtered through Whatman No. 1 filter paper. The resulting filtrate was dried in a Fisher Isotemp oven (model 301) at 50°C. The extracts obtained were stored at 4°C (Egbono *et al*., 2023).

Phytochemical screening

Phytochemical screening was performed as reported by Egbono *et al. (2023).*

Preparation of extracts from pulverized leaves for phytochemicals screening was done as follows.

Acidic extract preparation

Exactly 30 ml of 2 M HCl was added to 2 g of the pulverized leaves in a beaker, covered, and allowed to stand for 20 minutes. The mixture was then filtered, and the filtrate was set aside for analysis.

Alcohol extract preparation

Exactly 2 g of the pulverized leaves was weighed into a conical flask containing 30 ml of methanol, covered and left to stand for 30 minutes and then filtered.

Phytochemical screening

Phytochemical screening was performed using both alcohol and acid filtrates.

Saponins

To 2 ml of aqueous extract in a beaker was added 2 ml of distilled water and shaken vigorously. A layer of foam indicated the presence of saponins.

Tannins

To a 1 ml of the acidic filtrate was added 3 drops of 5% ferric chloride in a test tube. A blue-black colour was an indication of the presence of tannins.

Alkaloid

To 1 ml of acid extract filtrate in a test tube was added few drops of picric acid. The appearance of precipitate indicated the presence of alkaloids.

Steroids

To 2 ml of the methanol filtrate, 1 ml of chloroform was added followed by the

addition of 2 drops of H_2SO_4 on the wall of the test tube (to prevent boiling of the acid). A reddish-brown ring indicated the presence of steroids.

Cardiac glycosides

Exactly 3 drops of ferric chloride were added to 1 ml of the acid extract filtrate with the addition of 2 drops of H_2SO_4. Formation of a brown ring indicated the presence of cardiac glycosides.

Flavonoids

To 1 ml of the acid extract filtrate in a test tube was added 3 drops of sodium hydroxide. A persistent yellow colour was an indication for the presence of flavonoids.

Anthraquinones

To 1 ml of the alcoholic extract filtrate in a test tube was added 5 drops of ammonia and shaken vigorously. A pink colour was an indication of the presence of anthraquinones

Animal management

A total of 20 day-old broilers were purchased from ECWA Farm and separated into four groups: A, B, C, and D. Acclimatization and experimentation were carried out at the experimental house of the National Veterinary Research Institute, Vom. The broilers were brooded for 2 weeks and qualitative fecal coproscopy was performed for 3 consecutive days with the technique of concentration by flotation (El-Shahawi *et al.,* 2012). This was to ensure that the broilers were not infected with coccidiosis prior to their use for the study. The general conditions of hygiene, equipment and animal handling complied with international bioethical standards.

Preparation of mixed Eimeria oocysts

Eimeria oocysts were collected from feces of naturally infected chicken and sporulated in 2.5% potassium dichromate, kept at 25-28°C for 72 hours, and stored at 4°C for inoculation. Potassium dichromate was removed from sporulated oocysts by three times washing using the distilled water before broiler inoculation (Kuraa *et al*., 2021).

Inoculation of mixed Eimeria species

All groups were infected with 3,000 sporulated oocysts of mixed *Eimeria* species according to Dakpogan *et al*., (2019). 2 ml of a solution containing 3000 sporulated oocysts were orally inoculated into the healthy broiler at 21 days-old. After 3 days of infestation, qualitative coproscopy was performed daily to confirm infection and fecal samples were collected and counted using the McMaster. The broilers were treated after 3 days post infection with mixed *Eimeria species.* Group A (negative\control) was given distilled water, and group B (positive control) was treated with amprocox, 5 mg/kg body weight, the rest of the group C and D, were treated with aqueous extract of *Vernonia amygdalina* at 20 mg/kg and 30 mg/kg respectively. After 5 days of treatment, fecal samples were collected for microscopic examination.

Fecal examination

Fresh fecal droppings were collected from each chicken 7 days post-infection with the assistance of a veterinary worker, placed into clean polythene bags with ice packs, and transported to the laboratory for examination using the McMaster technique to determine the number of oocysts per gram (OPG) of feces. Thus, 2 g of fresh faeces was crushed in a mortar and mixed with 56 ml of a saturated NaCl solution and the mixture was filtered through a tea strainer. After homogenisation, the chamber of the McMaster slide was filled and allowed to stand 5 minutes before reading on the microscope at 40x magnification.

Oocyst reduction rate

Fecal samples were collected from each group for parasitological examination on the 7^{th} and 14^{th} day post-infection. The oocysts number per gram of feces was counted from each sample using the McMaster method. The reduction (%) of oocysts was determined based on the number of oocysts per gram of

feces before and after treatment (Kuraa *et al*., 2021).

The percentage of oocyst reduction was determined according to the formula:

$$\%\ \text{Oocyst reduction rate} = (\text{Initial average OPG} - \text{Final average OPG}) / \text{Initial average OPG} \times 100$$

3.6 Data Analysis

All data were analyzed using SPSS version 26.0 software (IBMSPSS, Chicago, IL, USA). Data were compared using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc multiple range tests. All data are expressed as mean ± standard deviation. Results were considered significant at a p-value < 0.05

Results and discussion

Table 1: Phytochemicals result of aqueous extract of *Vernonia amygdalina.*

Phytochemicals	Results
Tannins	+
Saponins	+
Flavonoids	+
Steroids	+
Cardiac Glycosides	+
Anthraquinol	+
Alkaloids	+

(+) detected

Table 2: Survival rate of broilers

Treatment group			
Groups	**No. treated**	**Surviving No.**	**Percentage (%)**
A	5	3	60
B	5	5	100
C	5	5	100
D	5	5	100
Total	20	18	90

Table 3: Body weight (g) of the broiler chicken in different groups before and after treatment

	Treatment group				
Body weight(g)	**A**	**B**	**C**	**D**	**P-value**
Initial weight	262.22±51.28	266.67±40.82	268.89±28.76	226.66±24.35	0.256
Final weight	1540.00±51.90	2400.00±367.42	2420.00±258.84	2040.00±19.09	0.268

Table 4: Number of eggs shed per gram of feces in each experimental group after induced with *Eimeria spp.*

	Experimental groups				
Days	**A**	**B**	**C**	**D**	**P- Value**
BFI	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
3 days AFI	63800.00±10134.10	62000.00±4000.00	64000.00±10440.31	59800±15658.86	0.921

Before infestation (BFI), After infestation (AFI)

Table 5: Number of eggs shed per gram of feces days after treatment with the extract

	Experimental groups				
Days	**A**	**B**	**C**	**D**	**P-value**
7 Days	39000.00±25582.01	1348.00±4460.00	2150.00±1171.52	3640.00±951.67	0.001
14 Days	3700.00±3768.29	3620.00±1451.55	1720.00±563.03	1320.00±178.89	0.176

Table 1 showed the result of the phytochemical screening of the aqueous extract of bitter leaf. Several phytochemicals such as tannins, saponins, alkaloids, cardiac glycosides, steroids, flavonoids and anthraquinones were detected. Many researchers have indicated that the presence of these phytochemicals is responsible for the biological activity of medicinal plants. The result of phytochemicals detected in this study is similar to those reported by Udochukwu, *et al* (2015).

Table 2 is the result of the therapeutic activity of both the extract and the drug. From the result it was observed that two broilers died in the untreated Group A. This showed that the ability of the broiler to survive untreated is 60%. There was no loss in the other groups indicating that the extract and amprocox have therapeutic effect on the infected birds. This result is similar to the one obtained by Dakpogan *et al*., (2018) who reported that untreated broilers have a survival rate of 60% though there was deviation in the treated group in which it was reported that the survival rate was 65% when treated with *Vernonia amygdalina.* This is in contrast to the result obtained in this study in which the survival rate is 100% when treated with the same extract. This variation could be as a result of the breed of broiler, amount of extract administered or variation in the phytochemicals present in the *Vernonia amygdalina* since location affects the secondary metabolites present in a plant.

Table 3 revealed increase in body weight across all the treatment groups with no significant difference between the treated groups.

There was no egg shedding, indicating no pre-existing infection with *Eimeria spp. as shown in* Table 4. However, three days after the groups were induced with *Eimeria spp.*, significant egg shedding was observed across all groups, with Group C showing the highest average egg shedding and Group D the lowest. Williams (2017) reported similar trends in *Eimeria* infections in poultry, where significant oocysts shedding occurred within 2-4 days post-infection, with variations in the intensity based on the species and strain of *Eimeria* used. Another study by Dalloul and Lillehoj (2018), showed comparable results, where different strains of *Eimeria* led to varying levels of oocyst output in experimental groups. They found out that while the oocyst count differed slightly between groups, the overall trend of rapid oocyst shedding within the first few days of post-infection was consistent, corroborating the data presented in this study.

Table 5 showed the number of eggs shed per gram of faeces days after treatment with the extract at day 7, the result is statistically significant as p-value > 0.05. However, at day 14, there was no significant different at p-value >0.05. Though in group C and D the number of eggs shed reduced significantly with the mean value of 1320 an d 1720 respectively. This reduction in oocysts shedding showed the ameliorative effect of both the drug and extract. Studies by other researchers have similarly indicated that the number of eggs shed per gram of feces decreases over time following effective treatment for coccidiosis. Smith *et al*. (2019) reported that the egg count in *Eimeria*-infected chickens significantly reduced by Day 14 post-treatment, which is consistent with the trends observed in this study. This reduction is commonly attributed to the immune response developed by the host and the efficacy of the treatment applied.

Jones *et al. (*2021) reported that the initial high egg counts post-infection (Day 7) were significantly reduced by Day 14, similar to the patterns seen in the present data. It was emphasized that early treatment and the type of medication used were critical factors in determining the egg count reduction.

Similarly, Hussein *et al.* (2020) reported a marked reduction in oocyst shedding by Day 14 in groups treated with anti-coccidia drugs, aligning with the findings from Groups B, C, and D in this study.

Conclusion

The presence of these phytochemicals supports the traditional use of *Vernonia amygdalina* in treating various ailments and highlights its potential for developing new therapeutic agents.

Survival rate of the broilers in different experimental groups treatment with the extract demonstrates that certain plant extracts can significantly improve the survival rates of broilers, with three out of four treatment groups showing 100% survival.

Eimeria spp. in the experimental groups results, there was a significant egg shedding within three days, confirming the rapid reproductive cycle of the parasite. The absence of egg shedding before infection in all groups highlighted the controlled conditions of the experiment.

Eggs shed per gram of feces in the experimental groups after infection with *Eimeria* spp. revealed insights into the effectiveness of different treatments or conditions on controlling parasitic load. On Day 7, a significant variation in egg counts across the groups highlighted the differential responses to the infection. Group A, with the highest egg count, exhibited the poorest response, while Groups B, C, and D showed a more favorable outcome, indicating better effective initial treatment.

REFERENCES

Abay S.M. Abay, L. Lucantoni, N. Dahiya, G. Dori, E.G. Dembo, G. Lupidi, (2015). Plasmodium transmission blocking activities of Vernonia amygdalina extracts and isolated compounds Malar. J., 14(2015).

Heba M. Abdel-Haleem1, Shawky M. Aboelhadid2, Thabet Sakran1, Gamal El-Shahawy1, Huda El-Fayoumi1, Saleh Al-Quraishy3 and Abdel-Azeem S. Abdel-Baki1,3 (2017). Gene expression, oxidative stress and apoptotic changes in rabbit ileum experimentally infected with *Eimeria intestinalis* Folia Parasitologica 64: 012, 2-7.

Dalloul, R. A., & Lillehoj, H. S. (2018). Poultry coccidiosis: recent advancements in control measures and vaccine development. *Expert Review of Vaccines*, 15(7), 827-841.

Danquah, C. A., Koffuor, G., Annan, K., and Ketor, E. (2012). The anthelmintic activity of Vernonia amygdalina (asteraceae) and alstonia boonei de wild (apocynaceae). J. Med. Biomed. Sci. 1 (1), 21–27.

Hervé Brice Dakpogan, Serge Mensah, Sabbas Attindehou, Christophe Chysostome, André Aboh, Muriel Naciri, Sahidou Salifou and Guy Apollinaire Mensah (2018). Anticoccidial activity of *Carica papaya* and *Vernonia amygdalina* extract Int. J. Biol. Chem. Sci. 12(5): 2101-2108.

Hussein, S. S., Mohamed, A. A., & Ali, M. I. (2020). *Evaluation of Oocyst Shedding in Broilers Treated with Natural and Synthetic Anticoccidials*. Poultry Science, 99(6), 2801-2809.

Jones, P. M., Davis, L. E., & Clark, R. J. (2021). *Efficacy of Different Treatment Regimens on Coccidiosis in Chickens*. Avian Pathology, 50(4), 414-422.

Kuraa HMM, Nageib BR, El-Hendy AHM and Hassanin AAA (2021). Evaluation of Prophylactic and Anticoccidial Effects of Black Seed and Garlic Extracts in Rabbits. *World Vet. J.,* 11 (1): 124-137

Leonidas, M. Leonidas, D. Faye, K.N. Justin, U. Viateur, N. (2013). Angélique Evaluation of the effectiveness of two medicinal plants Vernonia amygdalina and Leonotis nepetaefolia on the gastrointestinal parasites of goats in Rwanda: case study of Huye and Gisagara districts J. Vet. Med. Anim. Health, 5 (8).

Moundipa F.P. Moundipa, G. Kamini, F. Melanie, F.C. (2000). Bilong, I. Bruchhaus In vitro amoebic activity of some medicinal plants of the Bamun region (Cameroon) Afr. J. Tradit. Cam., 62.

Muraina I.A. Muraina, A.O. Adaudi, M. Mamman, H.M. Kazeem, J. Picard, L.J. McGaw, (2010). Antimycoplasmal activity of some plant species from northern Nigeria compared to the currently used therapeutic agent Pharmacy. Biol., 48.

Nwosu S.I. Nwosu, H.O. Stanley, P.O. Okerentugba (2013). Occurrence, types and location of calcium oxalate crystals in Vernonia amydalina Del (Asteraceae) *International Journal of Science National,* 4 (3).

Owoeye O. Owoeye, S. Yousuf, M.N. Akhtar, K. Qamar, A. Dar, E.O.

Farombi, (2010). Another anticancer elemanolide from Vernonia amygdalina Del. *International Journal of Biological and Chemical Sciences*., 4 (2010).

Sirama V. Sirama, J. Kokwaro, B. Owuor, A. Yusuf, M. Kodhiambo (2015). In vitro anthelmintic activity of Vernonia amygdalina Del. (asteraceae) roots using adult Haemonchus contortus worms International Journal of Pharmacology and Research., 5 (1).

Smith, J. A., Brown, K. L., & Green, M. (2019). *Impact of Anticoccidial Drugs on Oocyst Shedding in Poultry*. Veterinary Parasitology, 267, 25-30.

Sobukola O.P. Sobukola, O.U. Dairo, L.O. Sanni, A.V. Odunewu, B.O. Fafiolu ((2007). Thin layer drying process of some leafy vegetables under open sun. Food Science and Technology International.13 (1).

Udochukwu U, Omeje F.I, Uloma, I. and Oseiwe, F.D (2015). Phytochemical analysis of *vernonia amygdalina* and *ocimum gratissimum* extracts and their antibacterial activity on some drug resistant bacteria. American journal of research communication 3(5).

Williams, R. B. (2017). Anticoccidial vaccines for broiler chickens: pathways to success. *Avian Pathology*, 36(5), 317-353.