EFFECT OF METHANOLIC EXTRACT OF *Phyllanthus amarus* LEAVES ON CONTROL OF COCCIDIOSIS IN BROILER CHICKENS

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ABSTRACT
This study investigated the effect of Methanolic Extract of Phyllanthus amarus Leaves (MEPAL) in the control of coccidiosis in broiler chickens. The objectives were to examine the presence of coccida oocyst in the faecal samples of broiler birds treated with MEPAL and find out the effects of MEPAL on the blood profile of Eimeria-infected broiler chickens. A total of 150-day-old chicks were randomly allotted into 5 treatments and 3 replicates (10 birds each) in a completely randomized design experiment. The birds were fed ad libitum without coccidiostat. When they started showing clinical signs of coccidiosis at three weeks of age, they were treated with varying dosages of MEPAL for seven consecutive days. T1 received 5 ml, T2 received 10 ml, T3 received 15 ml, T4 were treated with Embazin-Forte while T5 did not receive any treatment (control). Results showed that 10 ml dosage of MEPAL significantly ($P < 0.05$) reduced coccidia oocyst counts (insert value) across 7-day post-medication period, showing similar effectiveness to the commercial anticoccidial drug Embazin-Forte. Haematological investigation indicated significant differences ($P < 0.05$) in packed cell volume (insert value) and monocyte levels (insert value) across the treatment groups. Moreover, the serum biochemistry analysis revealed that broiler chickens treated with 15 ml of MEPAL had the highest ($P < 0.05$) creatinine levels (3.00 mg/dL), while those treated with 5 ml MEPAL had the lowest (1.33 mg/dL). It was concluded that 10 ml MEPAL may be optimal for the control of coccidiosis comparable to commercial Embazin-Forte.
Keywords: Coccidiosis, Coccidia oocyst, Methanolic extract, *Phyllanthus amarus*, *Eimeria spp*

INTRODUCTION

Coccidiosis is an infective disease caused by *Eimeria* species of the Phylum apicomplexa. Over 1000 species of Eimeria have been reported to infect different host animals such as chicken, duck, turkey, cattle, rabbit, sheep and domestic dog and cat (Blake 2015). *Eimeria* oocyst is the main cause of coccidiosis which get into chickens via ingestion of food, water and litter contaminated with oocyst (Shivaramaiah *et al*. 2014). Coccidiosis infection lead to tissue damage associated with oxidative stress and lipid peroxidation, diarrhoeal hemorrhage, poor growth, increased susceptibility to other disease agents, and in severe cases, mortality (El-Shall *et al*., 2022). Anticoccidial drugs available for use finely or various combination are amprolium, clopidol, diclazuril, ethopabate halofuginanone and ionophores (monensin, lasalocid, narasin, maduramicin, nicarbasin, robenidine), and sulphaquinoxaline (Abebe and Gugsa, 2018).

However, continuous use of anticoccidial drugs leads to increased incidence of drug-resistant strain development which results in reduced activity of the drug against the causative agent. Thus, the use of natural alternatives to anticoccidial drugs is considered an attractive way to combat coccidiosis. The anticoccidial properties of natural herbal products (or their extracts) have been investigated. Habibi *et al*. (2016) reported that herbal extracts were effective in control of coccidiosis caused by the *E. tenella* infection; Ishaq *et al*. (2022) described the anticoccidial activity of ethanolic leaf extract of *Citrus aurantium* L.; while Hembesha *et al*. (2023) investigated the prophylactic anticoccidial effects of methanol extracts obtained from *Ganoderma lucidum*, *Vernonia amygdalina* Leaves and *Vitellaria paradoxa* Stem Bark. Furthermore, according to Rabiu *et al*. (2021), 100 mg/ml methanolic leaf extract of *Lannea schimperi* possess anticoccidial activity on experimentally induced *Eimeria tenella* in broiler chickens while Hamayun and Fazeel (2021) who evaluated the anticoccidial activity of methanolic extract of *Ricinus commun*is concluded that it can be used as a prophylactic and therapeutic agent at local and regional levels for the control of coccidiosis in broilers.

Phyllanthus amarus is a small erect tropical herb that grows to a height of 10 – 60 cm. It is an annual and widespread throughout the tropics and subtropics. It is found in sandy regions as a weed in cultivated and waste lands (Dabanka and Otchere, 2022). Owing to its immense medicinal properties, this plant has been valued in many countries for a variety of ailments. Verma *et al*. (2014) reported that the aqueous extract of *Phyllanthus*

amarus demonstrates potent anticancer, antiamnesic, antioxidative, antimicrobial, anti-inflammatory, antidiabetic, antifertility, antiviral and cardioprotective activities. Therefore, this study aims at evaluating the effect of methanolic extract of *Phyllanthus amarus* leaf (MEPAL) on the control of coccidiosis in broiler chickens.

MATERIALS AND METHODS

Experimental site

The research was carried out at the Poultry section of the Teaching and Research Farm of Federal University of Technology Minna, Niger State. Analyses were done at Animal Production Laboratory of the university located at Gidan Kwano along Minna-Bida Road, Minna, Niger State, Nigeria.

Preparation of *Phyllanthus amarus* extracts

Leaves of *Phyllanthus amarus* were harvested from the Niger State Agricultural and Mechanization Development Authority (NAMDA) demonstration farm in Minna. Fresh leaves were harvested, washed with distilled water and dried under shade for one week. The dried leaves were ground to fine powder using an electric grinding machine (Sonik Model SB-464). The fine powder was placed in a thimble made from thick filter paper which was loaded unto the main chamber of the soxhlet extractor. 250 ml of methanol as solvent was added to a round bottom flask attached to the soxhlet extractor and condenser. The solvent was heated and as it boiled, its vapour raised up and was condensed by the condenser. The condensate then dripped into the reservoir containing the thimble. Once the level of solvent reached the siphon, it poured back into the flask and the cycle repeated. Embazin-Forte was the conventional anti-coccidia drug used as control.

Source of birds

A total of 150-day old Agritech chicks were purchased from Ibadan and used for the experiment.

Housing and management

A week to the arrival of birds at the experimental site, the pen with the cages were thoroughly washed and cleaned using disinfectant (Lysol) and allowed to dry for a week. Wood shavings were used as floor litter and laid to $5cm^3$. On arrival of the chicks, anti-stress solution (mixture of water, glucose and multivitamin) was served as well as normal feed (Starter Top Feed, 22% CP, 2800 kcal/kg) and borehole water *ad libitum*. Routine vaccinations (Newcastle disease vaccine (NDV) i/o, Lasota and Infectious bursal disease (IBD) were administered accordingly during the two weeks of acclimatization. Anticoccidial medication was not included in the vaccination schedule. Also, heat was provided for the birds, day and night during this period. After acclimatization, the birds were allocated to five treatments in a completely randomized design. They were replicated thrice with 10 birds per replicate. Measured quantity of Starter Top Feed (0 – 4 wks old) and Finisher Top feed (5 wks and above, 19% CP, 3200 kcal/kg) were given by 7 am and 5 pm daily whereas clean borehole water was supplied *ad libitum* throughout the experimental duration of 6 weeks. When the birds started showing clinical signs of coccidiosis at Week 4, Methanolic Extract of *Phyllanthus amarus* Leaf (MEPAL) treatment was administered to them.

Treatment and experimental design

Methanolic Extract of *Phyllanthus amarus* Leaf (MEPAL) was measured at 5 mls, 10 mls and 15 mls respectively and added to one litre of drinking water. This was given to the birds to drink *ad libitum*. However, prior to that, the birds were given routine measured feed without water to make them thirsty and create the urge to drink the medicated water. When it was certain that all the birds had taken enough, it was replaced with clean borehole water. This was repeated for three consecutive days. The experimental dosing and grouping are as follows:

T1 = Birds treated with 5 ml of Methanolic Extract of *Phyllanthus amarus* Leaf (MEPAL)
T2 = Birds treated with 10 ml of Methanolic Extract of *Phyllanthus amarus* Leaf (MEPAL)
T3 = Birds treated with 15 ml of Methanolic Extract of *Phyllanthus amarus* Leaf (MEPAL)
T4 = Birds treated with Embazin-Forte (Positive control)
T5 = Birds not treated (Negative control)

Data collection

Oocyst count

McMaster counting technique was used to determine oocyst count according to the method described by Long *et al* (1976). At Week 4 when the birds started showing clinical signs of coccidiosis, a day before administration of experimental medication, fresh faecal samples were collected from all the five treatments and three replicates making a total of 15 samples in order to determine oocyst count. Faecal samples were subsequently collected for 7 consecutive days post-medication, making a total of 8 days in which faecal samples were collected and oocyst count determined. On a daily basis, 10 g of faeces was weighed per sample and mixed with 30 ml of saturated

Sodium Chloride (NaCl) floatation fluid. The resulting fluid was homogenized by mixing using stirring rod and labelled accordingly. Next, the faecal suspensions were passed through a fine sieve to remove large debris. The filtrates were collected in clean beakers. For each sample, a pipette was used to fill the two chambers of McMaster counting slide with the filtrates. The slide was allowed to sit for 2 minutes to allow the oocysts rise to the top and settled on the grid under the coverslip. The McMaster slide was then placed on a microscope

stage. The observation was first viewed using 10x and later reviewed under 40x for clear detection of the *Emeria* oocyst. They appeared as round or oval structures with clear walls which distinguished them from other debris. These were counted for both chambers of the McMaster slide for each sample. Thereafter, Oocysts per gram was computed using the formula below:

$$OPG = \left(\frac{Total\ Oocysts\ counted\ in\ both\ chambers}{Total\ grams\ of\ faeces\ used}\right) x\ Dilution\ factor$$

Where:
OPG = Oocysts Per Gram
Dilution factor = 50

Blood sample collection

2 ml of blood was collected through the wing venepuncture from two chickens each from replicate of individual treatments using a sterile syringe and needle; and put in ethylene diamine tetra-acetic acid (EDTA) treated-bottles to prevent clotting. The samples were quickly stored in an ice box using icepacks, and transferred to the laboratory for haematological analysis, within three hours post-sampling. Packed Cell Volume (PCV) was determined using the microhematocrit method described by Goldenfarb *et al.* (1971). The values obtained were expressed as a percentage of the total blood volume. Red Blood Cell (RBC) and White Blood Cell (WBC) counts were performed using a haematology analyser. Haemoglobin (Hb) concentration was determined using an Automated Veterinary Haematological Analyser. The MCV and MCHC were evaluated and analysed, and their values were expressed in g/ml of blood.

Fresh blood samples (2 ml) were collected through the wing venepuncture of two chickens each from each replicate using a sterile syringe and needle for serum biochemical analysis. Serum was obtained by centrifuging blood samples at 3,000 rpm for 10 minutes to separate serum from cells. Serum urea, sodium, potassium, creatinine, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase and total protein analyses were carried out according to the methods described by Chauhan and Agarwal (2006).

Data Analysis

The data were analysed using IBM Statistical Package for Social Science (SPSS version 23). Differences in means within and among treatment groups were tested using one-way ANOVA. Mean separation was done using Duncan Multiple Range Test. Probability value of 0.05 was considered as significant level of difference between two or more mean values.

RESULTS AND DISCUSSION

Presence of Coccidia Oocyst in Faecal Samples of MEPAL Treated Broiler Chickens

The presence of coccidia oocyst in faecal samples of broiler chickens treated with methanolic extract of *Phyllanthus amarus* Leaf (MEPAL) is presented in Table 1. No significant differences ($p>0.05$) were observed among the treatments on Day 1 (pre-medication). On Day 2 (first day post-medication), the faecal oocyst count (insert value) of the control group (T5) was significantly higher ($P < 0.05$) than those treated with various

concentrations of MEPAL (T1, T2, T3(insert value) and Embazin-Forte (T4) (insert value). From Day 3 onward, the oocyst counts in the control group remained consistently and significantly higher ($P < 0.05$) compared to all treatment groups.

Among the treatment groups, broiler chickens treated with 10 ml MEPAL and Embazin-Forte were statistically similar ($p>0.05$) in their oocyst count reductions, both showing the lowest oocyst counts across 7-day post-medication period, indicating comparable efficacy. Similarly, broiler chickens treated with 5 ml MEPAL and 15 ml MEPAL were statistically similar in their effects, though their oocyst counts were significantly higher ($P < 0.05$) than those treated with 10 ml MEPAL and Embazin-Forte By Day 8, the oocyst counts in all treated groups (T1, T2, T3, T4) was significantly lower ($P < 0.05$) than the oocyst counts of those in the control group (T5). These findings demonstrate that MEPAL, particularly at the 10 ml concentration, is a viable natural alternative to conventional anticoccidial drugs like Embazin-Forte. Meanwhile, the highest dosage of MEPAL (15 ml) exhibited a slight reduction in efficacy, suggesting the potential for diminishing returns at higher concentrations.

This finding is in line with Dauda *et al*. (2023) who indicated that different concentrations of *Phyllanthus amarus* ethanol leaf extract have anticoccidial activities as evidenced by their ability to significantly ($p < 0.05$) decrease the growth and development of oocysts to become the infective stage of *Eimeria* (sporozoites). The significant reduction in oocysts excretion induced by *Phyllantus amarus* could be attributed to its chemical compounds content such as phyllantine and hypophyllantine (Kushwaha *et al*., 2013). Dakpogan *et al*. (2019) studied the effect of *Phyllanthus amarus*, *Jatropha curcas* and *Piliostigma thonningii* leaf extracts on experimental chicken coccidiosis. It was discovered that *Phyllantus amarus* leaf extract was the most effective anticoccidian among the three medicinal plants used in the study in terms of reducing number of oocysts per gram in faeces with a reduction rate of 87%.

Table 1: Coccidia oocyst in faecal samples of infected broiler chickens treated with MEPAL

Parameters (oocysts/gram)	T1	T2	T3	T4	T5	SEM	P-Value	LS
Day 1	1306.66	1230.00	1310.00	1256.66	1266.66	21.33	0.786	NS
Day 2	1167.00^{a}	1062.00^{a}	1170.00^{a}	1086.00^{a}	1448.33^{b}	41.42	0.002	*
Day 3	1029.33^{b}	847.50^{a}	1032.00^{b}	867.50^{a}	1604.00^{c}	75.76	0.000	*
Day 4	886.66^{b}	648.00^{a}	896.00^{b}	664.00^{a}	1679.16^{c}	102.21	0.000	*
Day 5	748.00^{b}	515.00^{a}	756.00^{b}	528.33^{a}	1755.00^{c}	123.68	0.000	*
Day 6	547.50^{b}	360.50^{a}	558.00^{b}	369.83^{a}	1831.50^{c}	149.14	0.000	*
Day 7	293.33^{a}	206.00^{a}	302.50^{a}	211.33^{a}	1976.83^{b}	77.78	0.000	*
Day 8	175.00^{a}	95.00^{a}	180.00^{a}	97.66^{a}	2144.16^{b}	215.27	0.000	*

a,b,c = means in the same row with different superscript are significantly different ($P < 0.05$);
T1 = 5 ml methanolic extract of *Phyllantus amarus* leaf; T2 = 10 ml methanolic extract of *Phyllantus amarus* leaf; T3 = 15 ml methanolic extract of *Phyllantus amarus* leaf; T4 = positive control using Embazin-Forte; T5 = negative control

Haematological parameters and serum biochemistry of MEPAL Treated Broiler Chickens

The haematological parameters of broiler chickens treated with MEPAL in this experiment is shown in Table 2. There was a significant statistical difference ($P < 0.05$) in packed cell volume and monocytes across the treatments. Infected broiler chickens that were untreated (control) has recorded that highest values of PCV (27%) which were significantly different from PCV of broiler chickens treated with 10 ml MEPAL (21.86 %), 15 ml MEPAL (22.66 %) and Embazin-Forte (22 %). There was no statistical difference ($P > 0.05$) between the mean values of PCV of broiler chickens fed 5 ml MEPAL with those of other treatments. This result agrees with Nwankpa *et al*. (2014) and Sakthi *et al*. (2017). According to Vivian *et al*. (2015), the elevated PCV value in untreated broilers suggest a physiological response to infection, possibly as a compensatory mechanism for the parasitic stress caused by coccidiosis. In contrast, the lower PCV values in treated groups reflect reduced disease burden due to the therapeutic effects of MEPAL and Embazin-Forte.

Similarly, broiler chickens treated with 10 ml MEPAL and Embazin-Forte had the lowest mean values of monocytes (2.00 cells/µL) which was significantly different ($P < 0.05$) from the mean values of monocytes of untreated broiler chickens (3.00 cells/µL), indicating a potential immune-modulating effect. There was no

statistical difference between the mean values of monocytes of broiler chickens treated with 5 ml and 15 ml MEPAL (2.66 cells/µL each) with those of other treatments. On the other hand, haemoglobin (insert value), white blood cell count (insert value), mean corpuscular volume (insert value), mean corpuscular haemoglobin (insert value), mean corpuscular haemoglobin concentration (insert value), neutrophils (insert value), lymphocytes (insert value), and basophils(insert value) did not show any statistical difference ($P > 0.05$) across the treatments. These findings are in line with Unigwe *et al*. (2022) who discovered that monocytes were statistically significant across treatments. On the other hand, the findings of this study differ from Hidanah *et al*. (2018) who found significant differences ($P < 0.05$) in the eosinophils, basophils, heterophils and lymphocytes among treatment groups.

The serum biochemistry of broiler chickens treated with MEPAL in this experiment is shown in Table 3. It shows that broiler chickens treated with 15 ml MEPAL had the highest mean creatinine value of 0.77 mg/dL which was significantly different ($P < 0.05$) from the mean creatinine values of birds treated with Embazin-Forte (0.473 mg/dL), 5 ml MEPAL (0.466 mg/dL) and 10 ml MEPAL (0.560 mg/dL). Broiler chickens in control group had mean creatinine value of 0.630 mg/dL which was statisticaaly similar to those of other treatments.

This suggests that higher doses of MEPAL (15 ml) may affect kidney function, as elevated creatinine levels can indicate increased strain on the kidneys or reduced kidney efficiency. Naseem *et al*. (2018) stated that high serum creatinine in broilers indicates severe kidney damage and significant injury to the renal tubules, which negatively affects their overall health and growth performance. Thus, lower doses of MEPAL (5 ml) appear to have less impact on kidney and renal function, similar to Embazin-Forte.

Table 2: Haematological Parameters of MEPAL treated broiler chickens

Parameter	T1	T2	T3	T4	T5	SEM	P-Value	LS
Haemoglobin	8.067	7.233	7.533	7.300	7.300	0.300	0.932	NS
Packed Cell Volume	24.33^{ab}	21.86^{a}	22.66^{a}	22.00^{a}	27.00^{b}	0.671	0.047	*
Red Blood Cell Count	3.40	3.23	3.36	3.33	3.70	0.057	0.079	NS
White Blood Cell Count	21.00	16.33	17.66	17.00	25.33	1.202	0.068	NS
MCV	60.30	60.73	60.16	60.55	60.40	0.101	0.499	NS
MCH	20.00	20.10	20.00	20.06	20.06	0.023	0.717	NS
MCHC	33.10	33.03	33.16	33.16	33.16	0.034	0.769	NS
Neutrophils	34.00	32.33	32.66	32.33	36.66	0.616	0.097	NS
Lymphocytes	23.00	21.66	22.33	22.00	25.33	0.505	0.130	NS
Monocytes	2.66^{ab}	2.00^{a}	2.66^{ab}	2.00^{a}	3.00^{b}	0.133	0.024	*
Basophils	1.000	0.667	1.000	0.667	1.000	0.090	0.580	NS

a,b,c = means in the same row with different superscript are significantly different ($P < 0.05$);
T1 = 5 ml methanolic extract of *Phyllantus amarus* leaf; T2 = 10 ml methanolic extract of *Phyllantus amarus* leaf; T3 = 15 ml methanolic extract of *Phyllantus amarus* leaf; T4 = positive control using Embazin-Forte; T5 = negative control; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration

Table 3: Serum Biochemistry of MEPAL treated broiler chickens

Parameter	T1	T2	T3	T4	T5	SEM	P-Value	LS
Urea	5.633	6.667	7.300	6.200	6.667	0.241	0.274	NS
Sodium	129.3	144.0	143.3	130.6	142.6	2.607	0.161	NS
Potassium	3.600	4.033	4.500	4.067	4.667	0.143	0.115	NS
Creatinine	0.466^{a}	0.560^{a}	0.770^{b}	0.473^{a}	0.630^{ab}	0.036	0.014	*
Aspartate Aminotransferase	255.5	266.6	244.4	233.3	277.7	7.744	0.433	NS
Alanine Aminotransferase	11.66	11.33	12.00	10.33	11.66	0.305	0.530	NS
Alkaline Phosphatase	142.8	247.6	252.3	180.9	252.3	19.54	0.262	NS
Total Protein	4.203	4.516	4.143	3.826	4.303	0.148	0.741	NS

a,b,c = means in the same row with different superscript are significantly different ($P < 0.05$);
T1 = 5 ml methanolic extract of *Phyllantus amarus* leaf; T2 = 10 ml methanolic extract of *Phyllantus amarus* leaf; T3 = 15 ml methanolic extract of *Phyllantus amarus* leaf; T4 = positive control using Embazin-Forte; T5 = negative control

The results imply that while MEPAL is effective in managing coccidiosis, caution may be needed at higher dosages to avoid potential kidney stress. Lower doses may offer a safer alternative, providing therapeutic benefits without negatively affecting kidney health. This result differs from Onwujiariri *et al.* (2019) who reported that all blood parameters measured (albumin, globulin, urea, glucose and total protein) did not show any significant difference among treatments. This could be due to variation in breeds of broiler chickens. The authors made use of Sayed broilers while the present study uses Agritech broilers.

CONCLUSION

The administration of MEPAL, particularly at a 10 ml dosage, effectively reduced coccidia oocyst counts, showing similar efficacy to the commercial anticoccidial drug Embazin-Forte. Significant differences were observed in packed cell volume, monocytes and creatinine of coccidiosis infected broiler birds treated with MEPAL. Based on the significant reduction in oocyst count observed in broilers treated with 10 ml MEPAL, this dosage should be considered optimal for therapeutic use. It provides effective coccidiosis control comparable to Embazin-Forte, with minimal side effects. In addition to its anticoccidial activity, significant differences in packed cell volume, monocytes, and creatinine suggest that MEPAL also influences the immune and renal responses of broiler chickens which could make it effective in controlling gumboro and Newcastle diseases.

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