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Anticoccidial effect of hydromethanol leaf extract of *Tetrapleura tetraptera* in broiler chickens experimentally infected with *Eimeria tenella* oocysts

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Abstract

The anticoccidial effects of hydromethanol extract of *Tetrapleura tetraptera* leaf on broiler chickens infected with *Eimeria tenella* oocysts was investigated in this study. The leaves of *Tetrapleura tetraptera* were sourced, identified, washed, air dried, ground and extracted using hydromethanol. A total of 60, two weeks old Cobb 500 broiler chicks used for the study were divided into six experimental groups of ten birds each. Group 1 was the normal control (uninfected/untreated), Group 2 was the negative control (infected/untreated), Group 3 received amprolium (125 mg/L) and Groups 4, 5 and 6 received *Tetrapleura tetraptera* leaf extract (TTLE) at 250, 500 and 1000 mg/L of drinking water, respectively. There was significantly ($p < 0.05$) lower faecal oocyst count of TTLE (5566.67±898.77 – 2400.00±378.55 for TTLE at 250 mg/L) and amprolium (3400.00±550.71 – 1060.00±70.24) treated broilers when compared with the faecal oocyst count of the infected/untreated (9333.33±145.57 – 10000.00±115.47) group from days 13 to 18 post infection. The TTLE and amprolium treated groups showed a

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significant ($p < 0.05$) increase in the haemoglobin (Hb) levels of the treated broiler groups (12.53±0.70, 12.80±0.20, 13.07±0.60 and 13.13±0.29 g/dl, respectively) and packed cell volume (PCV) (27.67±3.28, 29.67±0.90, 30.67±3.28 and 31.67±1.45%, respectively). Feed conversion ratio (FCR) was significantly improved in TTLE, 250 mg/L (1.90±0.03) and TTLE, 1000 mg/L (1.58±0.02) over the infected/untreated (2.06±0.02). This study suggests that TTLE is a promising natural alternative to synthetic anticoccidial drugs.

Keywords: Anticoccidial activity, Broiler chicken, Coccidiosis, Hydromethanol, *Tetrapleura tetraptera*

Introduction

Coccidiosis is a parasitic disease affecting the intestinal tract of animals, primarily caused by protozoan parasites of the genus *Eimeria*. In poultry, the most pathogenic species is *E. tenella*. The severity of the disease depends on the *Eimeria* species involved, the number of oocysts ingested, and the host's immune status. Effective control and prevention measures include good sanitation, use of anticoccidial drugs, and vaccination. Historically, synthetic coccidiostats and coccidiocidals have been the mainstays of coccidiosis treatment and control. But concerns about drug-resistant *Eimeria* strains and the rise in drug residues of chicken products which cause health risks to consumers (Peek & Landman, 2011) have prompted researchers to look for alternative approaches to control the disease, such as using natural remedies (Chapman, 2014).

Tetrapleura tetraptera, locally known as 'Uhiokiriho' in South Eastern Nigeria belongs to the family *Fabaceae*. It is also known as African porridge fruit (Nweze *et al.,* 2011). *Tetrapleura tetraptera* is known for its diverse therapeutic properties, encompassing antimicrobial, antioxidant, antidiabetic, anti-inflammatory and cardiovascular health. A study by Adejumo *et al.* (2021) demonstrated that broilers fed diets supplemented with *T. tetraptera* extract had significantly lower *Eimeria* oocyst counts and improved gut health compared to those given standard feed. According to Hascoët *et al.* (2025), chickens exposed to coccidiosis and administered phytogenic supplement had fewer coccidia oocysts in their faeces and less intestinal damage compared to untreated chickens. The plant-based supplement showed comparable effects to anticoccidial drugs in controlling coccidiosis, without affecting growth, feed intake, or survival. This implies that phytogenic supplements could be a natural and effective alternative to drugs for treating coccidiosis. Due to the sparse research and dearth of information on the effect of *T. tetraptera* on coccidiosis, this study aims to evaluate the anticoccidial property of the hydromethanol extract of *T. tetraptera* leaf in broiler chickens experimentally infected with sporulated *Eimeria* oocysts in south eastern part of Nigeria.

Materials and Methods

Collection and extraction of Tetrapleura tetraptera leaves

Tetrapleura tetraptera leaves were sourced from the forest reserve of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The leaves were identified by Dr. Nwajiobi Benson of Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, then washed and air dried under shade at ambient laboratory temperature. The dried leaves were ground with grinding machine to obtain a coarse powder, which was then soaked in hydromethanol for 48 hours and stirred intermittently. After 48 hours the extract was filtered and concentrated in hot air oven at 40°C. The *Tetrapleura tetraptera* leaf extract (TTLE) was stored in a refrigerator until used throughout the duration of the experiment.

Experimental chickens and their management

This study was carried out in the Poultry Section of the Veterinary Teaching and Research Farm of Michael Okpara University of Agriculture, Umudike. Sixty (60) Cobb 500 broiler chicks procured from Zartech hatchery were used for the study. They were housed on deep litter in a brooding pen with both kerosene stoves and electric bulbs used as sources of heat. The chicks were fed commercial (Ultima®) chick mash and water *ad libitum*. At two weeks old the birds were randomly divided into six groups and were subsequently reared to maturity.

Infection of the birds

Three (3) ml containing 3000 sporulated oocysts of *Eimeria tenella* collected from the Department of Veterinary Parasitology and Entomology, Michael Okpara University of Agriculture Umudike were inoculated *per os* into each bird in groups 2-6 at two

weeks old. The faecal samples were collected from the floor to count oocysts using Mc Master Oocysts Count Technique (MOCT) to confirm the establishment of the infection. Treatment of the birds commenced at three weeks of age i.e 1-week post inoculation. The pen of the uninfected-untreated group was cleaned and bedding changed every day to avoid natural infection.

Experimental design

The birds were divided into six experimental groups of 10 birds each. Group 1 birds (uninfected/untreated) served as the normal control and were given only drinking water and feed *ad libitum.* Group 2 was infected/untreated and served as negative control. Group 3, was infected/ Amprolium-125mg/L treated served as the positive control. Group 4 was infected and treated with 250 mg/L of TTLE. Group 5 was infected and treated with 500 mg/L of TTLE. Group 6 was infected and treated with 1000 mg/L of TTLE. All the treatments were administered in their drinking water for seven days during which daily feed intake and weekly body weight were recorded. At the end of the 7th week of the study, blood samples were collected from the broilers via the jugular vein into EDTA anticoagulant and plain bottles to evaluate the haematological indices and serum biochemical parameters, respectively.

Ethical consideration

Ethical conditions governing the conduct of experiments with life animals were strictly observed. The experimental protocol was approved by the Ethical Committee (MOUAU/CVM/REC/202529) of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Determination of oocyst count

Fresh faecal samples were collected from the floor of each pen with spatula into a plastic container and weighed to obtain 1g. The sample was mixed with a saturated sugar solution; the suspension was thoroughly homogenized using a spatula and filtered through a sieve. Then, 0.3 ml of the filtrate was loaded into a McMaster counting chamber using a pipette. The chamber was allowed to settle for 5-10 minutes to ensure oocysts floated into the grid. The slide was examined under a light microscope (10× or 40× objective). Oocysts within the grid area were counted. The total oocyst count per gram of faeces was calculated using the formula:

$$\text{Oocysts per gram (opg)} = \frac{\text{Total oocysts counted}}{\text{Volume of count}}$$

Measurement of haematological parameters

The manual haemocytometer method was employed in RBC and total WBC count; haematocrit method was used in PCV estimation while Hb concentration was estimated with cyanomethaemoglobin method using Drabkin's reagent. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) was calculated as described by Brar *et al.* (2000). Thin blood smear was made on clean dry and grease-free microscope slide from each of the collected blood sample in EDTA. The slides were air dried and stained with Giemsa stain as described by Brar *et al.* (2000). The slides were later examined with light microscope under oil immersion. The relative number of the neutrophils, lymphocytes, eosinophils, basophils and monocytes were estimated using the haemocytometer method.

Evaluation of biochemical parameters

A commercially available reagent kit (Randox Diagnostic Laboratories, United Kingdom) was used to evaluate the serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities as well as serum total protein, albumin, total cholesterol, and triglyceride. The assays were carried out as instructed by the manufacturer.

Carcass analysis

At the end of the 7th week of the experiment, three broilers were randomly selected from each group, weighed and sacrificed for carcass analysis. Sacrfice was by severing the jugular vein. The feathers were loosened by immersing in hot water followed by de-feathering.

The abdomen was cut open and viscera pulled out. The carcass was cut into parts (wing, back cut, breast, drum stick and thigh) and weighed. The weights of different carcass parts were expressed as percentage of the live weight.

Statistical analysis

The data obtained from the study, including oocyst count, body weight gain, feed conversion ratio and other parameters were analyzed using Statistical Package for Social Science (SPSS 2008), version 22. One-way analysis of variance (ANOVA) coupled with Tukey post-hoc test was used to compare the

differences between treatment groups. Values of $p < 0.05$ were considered significant.

Results

Effect of TTLE on growth parameters of broiler chickens infected with Eimeria tenella oocysts

The effects of TTLE on growth parameters of broiler chickens infected with *Eimeria tenella* oocysts is presented in Table 1. There was no significant difference ($p > 0.05$) in the final body weight of the TTLE (250, 500 and 1000 mg/L) and amprolium – 125 mg/L treated groups when compared with the infected/untreated group. For the weight gain, there was significant difference ($p < 0.05$) in TTLE (500 mg/L) when compared with infected/untreated group. There was also significant difference ($p < 0.05$) in the FCR of TTLE (250 and 1000 mg/L) treated groups when compared with infected/untreated group.

Effects of TTLE on live weight and carcass analysis of broiler chickens infected with Eimeria tenella oocysts

The effects of TTLE on carcass analysis of broiler chickens infected with *Eimeria tenella* oocysts is presented in Table 2. There was no significant difference ($p > 0.05$) in the live, defeathered, and dressed weights of the treatments when compared with infected/untreated group. The backcut weight of all the treatment groups was significantly ($p < 0.05$) lower than the infected/untreated group. The breast weight of TTLE (500 and 1000 mg/L) was not significantly ($p > 0.05$) different from the infected/untreated group. The drumstick of TTLE (250, 500 and 1000 mg/L) was significantly ($p < 0.05$) lower than the infected/untreated group.

Effects of TTLE on the faecal oocysts count of broiler chickens infected with Eimeria tenella oocysts

The faecal oocysts count across the six groups for days 7, 10, 13, 15 and 18 post infection is presented in Table 3. No oocyst was found in the faeces of the uninfected/untreated group throughout the duration of the experiment; while there was a gradual increase in the faecal oocyst count of the infected/untreated throughout the period of the study. The faecal oocyst counts of TTLE, as well as amprolium treated groups declined significantly ($p < 0.05$) from day 10 post infection up to the end of the experiment. There was also a significant ($p < 0.05$) decline in the faecal oocyst counts of TTLE and amprolium treated groups when compared with that of the infected/untreated group on days 13, 15 and 18 post infection.

Table 1: Effect of TTLE on growth parameters of broiler chickens infected with *Eimeria* spp.

Treatments	Initial wt (g)	Final wt (g)	Wt gain (g)	Feed intake (g)	FCR
Uninfected/untreated	691.03±2.72	2107.40±3.18	1440.90±0.47	4710.00±7.57*	3.17±0.09
Infected/untreated	839.90±2.48*	2341.33±2.57*	1500.53±1.56*	3107.77±6.83	2.06±0.02
Amprolium, 125 mg/L	691.03±1.40	2340.88±2.00*	1647.70±1.44*	4188.37±21.91*	2.54±0.02
TTLE, 250 mg/L	801.27±0.54*	2513.17±1.60*	1711.33±0.57*	3245.07±14.94	1.90±0.03*
TTLE, 500 mg/L	962.17±2.72*	2432.50±2.61*	1469.33±0.67	3263.00±18.39	2.20±0.03
TTLE, 1000 mg/L	789.47±2.17	2337.50±0.45*	1545.10±0.23*	2491.57±14.63	1.58±0.02*

*$p < 0.05$ when compared with the infected/untreated group; TTLE = *Tetrapleura tetraptera* leaf extract; FCR = Feed conversion ratio

Table 2: Effects of TTLE on live weight and carcass analysis of broiler chickens infected with *Eimeria* spp.

Treatments	Uninfected/ Untreated	Infected/ Untreated	Amprolium (125 mg/L)	TTLE (250 mg/L)	TTLE (500 mg/L)	TTLE (1000 mg/L)
Live wt (g)	1942.50±14.43	2141.50±38.39	2088.50±28.00	1958.00±24.29	1850.00±56.58	2207.00±27.81
Defeathered (%	95.77±1.21	93.23±2.02	97.01±1.54	93.47±0.60	93.73±0.39	95.04±0.28
Dressing (%)	72.33±1.98	74.11±0.60	73.13±2.07	68.21±1.27	73.95±0.38	72.32±0.48
Wing (%)	7.73±0.37*	6.25±0.09	7.78±0.12*	7.26±0.40*	7.98±0.22*	7.43±0.02*
Backcut(%)	11.24±0.20*	17.08 ± 0.53	11.78±0.05*	11.10±0.57*	15.20±0.62	12.39±1.15
Breast (%)	27.00±2.13	28.57±0.89	26.08±0.74*	23.73±0.58*	27.60±0.53	27.43±0.46
Drumstick (%)	10.57±0.11*	11.97±0.37	11.29±0.27	10.85±0.26*	10.22±0.03*	8.34±0.71*
Thigh (%)	13.00±0.21*	9.93±0.38	12.34±0.68*	11.45±0.53*	8.39±0.30*	11.47±0.66*

*$p < 0.05$ when compared with the infected/untreated group; TTLE = *Tetrapleura tetraptera* leaf extract

Table 3: Effects of TTLE on faecal oocyst count of broiler chickens infected with *Eimeria* spp.

Treatment	Day 7 PI	Day 10 PI	Day 13 PI	Day 15 PI	Day 18 PI
U/U	0.00±0.00	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
I/U	7433.33±744.61	8800.00±305.51	9333.33±145.57	9533.33±120.19	10000.00±115.47
Ampr (125 mg/L)	6200.00±378.60*	4933.33±417.60*	3400.00±550.71*	2266.67±317.98*	1060.00±70.24*
TTLE (250 mg/L)	7800.00±642.91	7300.00±680.69	5566.67±898.77*	3900.00±669.16*	2400.00±378.55*
TTLE (500 mg/L)	7900.00±971.25	5983.33±967.10*	3533.33±845.43*	2066.67±571.47*	1060.00±149.77*
TTLE (1000 mg/L)	8100.00±608.28	6566.67±491.00*	3333.33±480.75*	1366.67±272.77*	800.00±64.29*

*p<0.05 when compared with infected untreated group, TTLE = *Tetrapleura tetraptera* leaf extract, PI = Post infection, U/U = uninfected/untreated, I/U = infected/untreated, Ampr = Amprolium

Table 4: Effects of TTLE on blood parameters of broiler chickens infected with *Eimeria* spp.

Treatment	Uninfected/ untreated	Infected/ untreated	Amprolium (125 mg/L)	TTLE (250 mg/L)	TTLE (500 mg/L)	TTLE (1000 mg/L)
Hb (g/dL)	13.27±0.37*	11.13±0.29	13.13±0.29*	12.53±0.70*	12.80±0.20*	13.07±0.60*
PCV (%)	31.33±2.33*	22.00±1.15	31.67±1.45*	27.67±3.28*	29.67±0.90*	30.67±3.28*
RBC ($X10^6$/µL)	3.59±0.25*	2.49 ± 0.12	3.59±0.16*	3.12±0.35*	3.36±0.10*	3.50±0.36*
MCV (fL)	87.30±0.50	88.33±0.28	88.20±0.05	88.66±0.53	88.20±0.08	87.62±0.49
MCH (pg)	37.19±1.46*	44.82 ± 1.08	36.66±0.87*	40.71±2.20*	38.09±0.57*	37.78±2.02*
MCHC (g/dL)	42.62±1.88*	50.75±1.35	41.57±1.01*	45.95±2.73*	43.18±0.68*	43.15±2.52*
TWBC ($\times10^3$/µL)	20.40±0.53*	32.98 ± 2.04	24.65±1.09*	22.35±1.60*	21.93±0.66*	19.70±0.75*
Lymphocyte (%)	61.33± 1.45*	40.67± 0.88	55.33±1.45*	51.33± 0.88*	54.33± 0.33*	56.67± 0.33*
Heterophil (%)	31.00±0.58*	41.00±1.53	34.33±2.33*	39.00 ± 1.15	37.00±0.58*	36.33±1.20*
Monocyte (%)	5.33 ± 0.67	6.00 ± 0.58	4.67 ± 0.33	6.00 ± 0.58	6.00 ± 1.15	5.33 ± 0.67
Eosinophil (%)	2.33± 0.33*	11.67± 0.88	5.67± 0.67*	3.33± 0.33*	2.67± 0.67*	2.50± 0.50*

*p<0.05 when compared with infected untreated group, TTLE = *Tetrapleura tetraptera* leaf extract, Hb =haemoglobin, PCV = packed cell volume, RBC = red blood cell, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, TWBC = Total white blood cell

Effects of TTLE on haematological parameters and differential WBC count of broiler chickens infected with Eimeria tenella oocysts

The results of haematological parameters for Hb, PCV, RBC, MCV, MCH, and MCHC; and leucocytic profile is presented in Table 4. The TTLE (250, 500 and 1000 mg/L) and amprolium (125 mg/L) caused a significant ($p < 0.05$) increase in the Hb and RBC levels of the treated broiler groups when compared with the infected untreated broiler group. There was a significant ($p < 0.05$) decrease in the MCH and MCHC levels of the treated broiler groups when compared with the infected untreated broiler group. All doses of TTLE including Amprolium, 125 mg/L significantly reduced the total white blood cell (TWBC) when compared to the infected/untreated group, with the highest dose (1000 mg/L) showing the lowest TWBC. The TTE (500 and 1000 mg/L) produced a significant ($p < 0.05$) decrease in the heterophils and eosinophils count when compared to the infected/untreated group. The TTLE (250 mg/L) did not produce a significant difference ($p > 0.05$) in the heterophil count when compared to the infected/untreated group. The TTLE (250, 500 and 1000 mg/L) and amprolium (125 mg/L) caused a significant ($p < 0.05$) increase in the lymphocyte levels of the treated broiler groups when compared with the infected/untreated broiler group. The increase in TTLE groups was found to be dose-dependent with the highest dose (1000mg/L) producing the highest lymphocyte level while the lowest dose (250 mg/L) produced the lowest level.

Effects of TTLE on the serum biochemical parameter of broiler chickens infected with Eimeria tenella oocysts

The effects of TTLE on the total protein, albumin, globulin, ALP, ALT, ASP, cholesterol, triglyceride, high density lipoprotein (HDL), very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) of broiler chickens infected with *Eimeria* oocysts is presented in Table 5. The infected/untreated and the Amprolium 125 mg/L groups were not significantly different ($p > 0.05$) in their values for total protein, albumin, globulin ALP, and AST; but were significantly lower than the TTLE treated broiler chickens.

Table 5: Effects of TTE on the serum biochemical parameters of broiler chickens infected with *Eimeria* spp.

Treatments	Uninfected/ untreated	Infected/ untreated	Amprolium (125 mg/L)	TTLE (250 mg/L)	TTLE (500 mg/L)	TTLE (1000 mg/L)
Total protein (g/dl)	2.90±0.10*	2.74±0.01	2.73±0.08	2.56±0.01*	2.80±0.03	2.91±0.02*
Albumin (g/dl)	1.58±0.02*	1.39±0.04	1.33±0.03	1.49±0.02*	1.41±0.01	1.45±0.02
Globulin (g/dl)	1.32±0.10	1.35±0.04	1.40±0.10	1.07±0.01*	1.39±0.02	1.46±0.03
ALP (U/L)	113.88±0.08	113.34±0.23	113.43±0.25	114.15±0.08*	114.15±0.27*	114.24±0.09*
ALT (U/L)	7.20±0.09*	4.88±0.23	5.52±0.32	5.88±0.21*	5.40±0.52	5.75±0.20
AST (U/L)	87.70±0.75	87.05±2.63	94.85±6.38	111.75±1.13*	116.95±2.63*	157.90±2.25*
Cholesterol(mg/dl)	112.64±3.31*	97.23±3.55	89.05±1.20*	123.23±0.48*	99.88±2.51	95.31±1.82
Triglyceride(mg/dl)	127.30±3.74*	143.49±1.27	114.92±4.48*	123.49±1.14*	91.43±8.85*	116.51±0.32*
HDL (mg/dl)	51.71±1.49*	44.55±0.65	48.46±0.86	68.94±0.65*	46.18±0.86	45.85±2.46
VLDL (mg/dl)	25.46±0.75*	28.70±0.25	22.98±0.90*	24.70±0.23*	18.29±1.77*	23.30±0.06*
LDL (mg/dl)	35.47±3.40*	23.98±3.30	17.61±0.46	29.58±0.85	35.42±1.12*	26.15±4.28

*p<0.05 when compared with infected untreated group, TTLE = *Tetrapleura tetraptera* leaf extract, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase, HDL = High Density Lipoprotein, LDL = Low Density Lipoprotein, VLDL = Very Low Density Lipoprotein

The TTLE treated and amprolium treated groups produced values for triglyceride and VLDL that were significantly ($p < 0.05$) lower than the infected/untreated group.

Discussion

This study evaluated the anticoccidial effects of TTLE on broiler chickens. The impact of TTLE on growth in broilers challenged with *Eimeria* oocyst was obvious in this study. The negative effect was seen in the infected/untreated group where despite high feed intake, the weight gain was comparably small leading to a poor feed conversion ratio. The broilers in this group probably consumed more feed to compensate for metabolic inadequacies caused by infection. These findings align with Azzi *et al.* (2016) who reported that infection leads to impaired nutrient absorption and increased feed consumption without optimal weight gain. *Tetrapleura tetraptera* extract showed dose dependence on its positive effect on growth in this study. Broiler chickens administered TTLE (1000 mg/L) had the overall best feed conversion ratio. Though the infected/untreated group recorded the highest weights in some parts of the carcass such as the backcut and drumstick, overall, there was no significant difference ($p<0.05$) in the live weight, defeathered and dressing percentage.

The progressive increase in the faecal oocyst count of the infected/untreated group reflects the natural progression of coccidiosis (Onyeabor *et al.,* 2025). The infected/untreated group demonstrated that infection leads to a steady rise in oocyst count, confirming the pathogenicity of the parasite (Taylor *et al.,* 2022). The TTLE groups exhibited dose-dependent efficacy in controlling oocyst shedding. At 500 mg/L and 1000 mg/L, the reductions were comparable to or even better than Amprolium by Day 18, suggesting that TTLE could be a promising natural alternative. The lower dose (250mg/L) was effective but less potent than higher doses, indicating that efficacy depends on concentration. The anticoccidial activity of TTLE could be attributed to the presence of some bioactive compounds. Abii & Elegalam (2007) and Aladesanmi (2007) reported that bioactive compounds in *T. tetraptera* include alkaloids, tannins and saponins. These compounds are known to have antimicrobial, antiviral and antiparasitic activities (Aladesanmi, 2007; Kemigisha *et al.,* 2018).

Coccidiosis like most parasitic infections often induces anaemia and leukocytosis due to blood loss and immune system activation (Djokic *et al.,* 2021). The uninfected/untreated group, serving as the positive control, consistently showed high values for Hb, PCV and RBC; while the infected/untreated group had reduced Hb, PCV, and RBC levels, indicative of anaemia (Onyeabor *et al.,* 2025). The haematological values of higher doses of TTLE (500 mg/L and 1000 mg/L) were close to the value for the uninfected/untreated group, which infers that higher doses of TTLE has potential as a natural alternative or adjunct therapy to synthetic drugs. The elevated TWBC and eosinophils count in the infected/untreated group reflect an active immune response to the infection. The increase in eosinophils count suggests parasitic or allergic inflammation, while the leukocytosis indicates a systemic immune reaction. These findings align with the works of

Akinwale *et al.* (2018) and Abd El-Rahman *et al.* (2020), which identified leukocytosis and eosinophilia as hallmark responses to parasitic infections.

The infection significantly reduced total protein, albumin, and globulin levels in the infected/untreated group, indicating a possible physiological impact of infection on protein metabolism. Treatment with Amprolium did not significantly improve total protein and albumin levels compared to the infected untreated group. This suggests limited efficacy of Amprolium in restoring protein balance under the experimental conditions. But treatment with TTLE (1000 mg/mL) showed significantly higher values for total protein and globulin levels, indicating improved protein metabolism at this higher dose. This may be due to the ability of TTLE to mitigate oxidative stress and support liver protein synthesis (Ojo *et al.,* 2013).

The infected/untreated group shows the adverse effects of infection on lipid metabolism by exhibiting elevated cholesterol, triglycerides, LDL, and VLDL, and reduced HDL levels. Parasitic infections have been associated with dyslipidemia due to altered liver metabolism and function (Onyeabor *et al.,* 2025). The lipid profile of TTLE treatment groups, especially at 250 mg/L and 500 mg/L, showed a dose-dependent improvement in lipid parameters, with significant increases in HDLC and decreases in triglycerides and VLDLC. Amprolium showed some improvements but was less effective than TTLE at higher doses. For the liver enzymes, ALT is a key marker for liver health. The TTLE treatment has no hepatotoxic effect because ALT level is within the normal range for broiler chickens. This can be attributed to the bioactive compounds contained in TTLE which have antioxidant properties and can protect hepatocytes from damage caused by oxidative stress and inflammation (Iweala, 2012). The increase in AST with higher TTLE doses (especially 1000 mg/L) could indicate increased hepatic enzymatic activity due to detoxification in response to treatment.

In conclusion, hydromethanol leaf extract of *T. tetraptera* demonstrated significant dose-dependent anticoccidial efficacy comparable to amprolium in *E. tenella*-infected broilers, without adverse hematological or hepatic effects. The extract shows promise as a safe, plant-based alternative for managing poultry coccidiosis.

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Conflict of Interest

The authors declare that there is no conflict of interest.

References

Abd El-Rahman GI, Behairy A, Elseddawy NM, Batiha GES, Hozzein WN, Khodeer DM & Abd-Elhakim Y (2020). Saussurea lappa ethanolic extract attenuates triamcinolone acetonideinduced pulmonary and splenic tissue damage in rats via modulation of oxidative stress, inflammation, and apoptosis. *Antioxidants,* doi.10.3390/antiox9050396.

Abii TA & Elegalam A (2007). Investigation into the chemical composition of the Dry fruit of *Tetrapleura tetraptera* (Uhiokiriho). *Journal of Food Technology,* **5**(3): 229 – 232.

Adejumo DO, Olaniyi AO, Abioye SA, Ekanem AP, Francis G, Hashemi SR, Min BR, Oluwafemi RA, Peek HW, Shirley MW, Sofowora A, Williams RB, Windisch W, Schedle K, Plitzner C, Kroismayr A, Steentoft M, Osei-Tutu P, Nketiah K & Faniyan J (2021). *Tetrapleura tetraptera* extract in enhancing broiler immunity and gut health. *Animal Nutrition,* **7**(4): 586-594.

Aladesanmi AJ (2007). *Tetrapleura tetraptera*: molluscicidal activity and chemical constituents. *African Journal of Traditional Complementary and Alternative Medicines,* **4**(1): 23–36.

Azzi A, Maydani SN, Maydani M & Zingg JM (2016). The rise, the fall and the renaissance of vitamin E. *Archives of Biochemistry and Biophysics*, doi.10.1016/j.abb.2015.11.010.

Brar RS, Sandhu HS & Singh A (2000). Veterinary Clinical Diagnosis by Laboratory Methods. Kalyani Publishers, New Delhi. Pp 146 – 150.

Chapman HD (2014). Milestones in avian coccidiosis research: A review. *Poultry Science,* **93**(3): 501-511.

Djokic V, Rocha SC & Parveen N (2021). Lessons learned for pathogenesis, immunology, and disease of erythrocytic parasites: *Plasmodium* and *Babesia*. *Frontiers in*

Cellular and Infection Microbiology, **11**: 685239.

Hascoët A, Torres-Celpa P, Riquelme-Neira R, Hidalgo-Olate H & Hidalgo-Olate H (2025). Evaluation of the effectiveness of a phytogenic supplement (Alkaloids and Flavonoids) in the Control of *Eimeria* spp. In experimentally challenged broiler chickens. *Animals*, doi.10.3390/ ani15060847.

Iweala EE (2012). Analgesic and hepatoprotective activity of methanolic leaf extract of *Ocimum gratissimum* (L.). *Research Journal of Medicinal Plant,* **6**(1): 108-115.

Kemigisha E, Owusu EO, Elusiyan CA, Omujal F, Tweheyo M & Bosu PP (2018). *Tetrapleura tetraptera* in Ghana, Nigeria and Uganda: household uses and local market. *Forests, Trees and Livelihoods,* doi.10.1080/ 14728028. 2018.1498027.

Nweze BO, Nwankwegu AE & Ekwe OO (2011). The Performance of Broiler Chickens on African Porridge Fruit (*Tetrapleura tetraptera*) Pod under Different Feeding Regimes. *Asian Journal of Poultry Science,* **5**(4): 144 – 149.

Ojo AO, Oloyede OI, Olarewaju OI & Ajiboye BO (2013). Evaluation of transaminases activity of aqueous extract of *Ocimum gratissimum* in the liver and kidney of Albino Rats. *International Journal of Biological & Medical Research,* **4**(4): 3650-3653.

Onyeabor A, Nweze EC, Ukwueze IJ, Ozioko CA, Uwabunkeonye IL, Igwe IR, Okey SN, Ifenkwe DC, Otuh PI, Gurama S, Onoja SO, Ani NV, Madubuike KG & Okorie-kanu CO (2025). Anticoccidial effects of methanol extract of *Ocimum gratissimum* (scent leaf) on broilers infected with *Eimeria* oocysts. *Journal of Sustainable Veterinary and Allied Sciences,* **7**(1): 92-99.

Peek HW & Landman WJM (2011). Coccidiosis in poultry: anticoccidial products, vaccines and other prevention strategies. *Veterinary Quarterly,* **31**(3): 143-161.

SPSS (2008). Statistical package for social sciences program. Version 22 for Windows, SPSS. Inc., Chicago.

Taylor J, Sakkas P & Kyriazakis I (2022). Starving for nutrients: anorexia during infection with parasites in broilers is affected by diet composition. *Poultry Science,* doi.10.1016/j.psj.2021.101535.