



Evaluation of the anti-coccidial effect of aqueous *Sacoglottis gabonensis* (Magnoliophyta, Humiriaceae) stem bark extract in broilers experimentally infected with mixed *Eimeria* species

Chukwuma P. Eze, Chukwunonso F. Obi, Idika K. Idika, and Chukwunyere O. Nwosu

University of Nigeria, Fac. of Veterinary Medicine, Dept. of Veterinary Parasitology and Entomology, Nsukka, Nigeria

Abstract

Aim of study: The aqueous *Sacoglottis gabonensis* stem bark extract (SGSBE) was evaluated for its anti-coccidial effects and toxicity in broiler chickens

Area of study: Nsukka, Nigeria

Material and methods: A hundred and nineteen, four-week old, broiler birds were used for this study. The toxicity of SGSBE was evaluated by administering graded doses of the extract once and for 21 days. The anticoccidial effect of SGSBE was determined using 25 birds arbitrarily divided into five groups (A-E) of five birds each. Groups A-D were orally infected with 200,000 sporulated mixed *Eimeria* oocysts while group E served as the uninfected control. Groups A and B birds were treated orally with SGSBE (200 mg/kg) once and daily for five consecutive days respectively while group C birds were treated with amprolium daily for five days. Birds in group D remained infected-untreated. The birds were observed for clinical signs, body weight changes, oocyst output, and some haemato-biochemical parameters.

Main results: Mild signs of toxicity were detected with mortality only in the group that received the highest dose of SGSBE following toxicity tests. Clinical signs of coccidiosis were observed following infection of the birds. Oocyst output, clinical signs and lesions were significantly reduced ($p < 0.05$) while body weight, survivability and haemato-biochemical indices of the birds were significantly improved ($p < 0.05$) in SGSBE treated groups. Moreover, five days consecutive treatment with SGSBE yielded better results.

Research highlights: The aqueous *S. gabonensis* stem bark extract is relatively safe and possesses anti-coccidial efficacy against mixed *Eimeria* infections in broiler chickens.

Additional key words: coccidiosis; oocyst count; toxicity; ethoveterinary medicine; poultry.

Abbreviations used: DPI (days post infection); HbC (haemoglobin concentration); PCV (packed cell volume); RBC (red blood cell count); SA (serum albumin); SGC (serum globulin concentration); SGSBE (*Sacoglottis gabonensis* stem bark extract); TLC (total leucocyte count); TSP (total serum protein).

Authors' contributions: CON conceived, designed and supervised the research. CPE, CFO and IKI conducted the experiments. IKI analysed the data. CFO wrote the manuscript. CON, IKI and CFO reviewed and edited the manuscript. All authors read and approved the manuscript.

Citation: Eze, PC; Obi, CF; Idika, IK; Nwosu, CO (2022). Evaluation of the anti-coccidial effect of aqueous *Sacoglottis gabonensis* (Magnoliophyta, Humiriaceae) stem bark extract in broilers experimentally infected with mixed *Eimeria* species. Spanish Journal of Agricultural Research, Volume 20, Issue 1, e0503. <https://doi.org/10.5424/sjar/2022201-18620>

Received: 10 Jul 2021. **Accepted:** 01 Feb 2022.

Copyright © 2022 CSIC. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) License.

Funding: The authors received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

Correspondence should be addressed to Chukwunonso Francis Obi: Chukwunonso.obi@unn.edu.ng; drfrancis.obi@gmail.com

Introduction

Coccidiosis is amongst the important fatal diseases of poultry and limits poultry production worldwide (Usman *et al.*, 2011). In the Nigerian poultry sector, the disease is the most consistently reported health problem (Jatau *et al.*, 2012) with many *Eimeria* species co-infecting birds (Agishi *et al.*, 2016). Avian coccidiosis control is principally by the use of anticoccidial drugs (Alhotan & Abudabos, 2019; Noack *et al.*, 2019) in ad-

dition to bio-security measures. However, the efficacy of these anticoccidial drugs is impaired by the emergence of drug resistant strains (Usman *et al.*, 2011). Other drawbacks to the use of synthetic anticoccidial drugs include prolonged withdrawal period, occurrence of drug residues and high cost of anticoccidial drugs (Chapman *et al.*, 2010; Ahad *et al.*, 2018; Noack *et al.*, 2019). Currently, botanicals and herbal drugs are very promising alternatives to synthetic anticoccidials for the treatment of avian coccidiosis.

Traditional veterinary care in most Nigerian rural communities forms the first level of the animal health care delivery system. Its dependence on botanicals ensures its affordability, availability, and popularity amongst local farmers. Several plants are used in folkloric medicine in most rural sub-Saharan African villages to treat many diseases of man and animals (Mbaya *et al.*, 2007; Muthamilselvan *et al.*, 2016). However, the safety and effectiveness of some of these plants are uncertain (Nwosu *et al.*, 2004; 2008).

Sacoglottis gabonensis (Magnoliophyta: Malpighiales: Humiriaceae) is found in the tropical rain forest regions of West Africa and America. In Nigeria, the plant is locally known as 'nche' in Igbo, 'atale' in Yoruba, 'ugu' in Bini, 'ndai' in Efik and 'edat' in Boki and Ibibio languages (Nwosu *et al.*, 2001). The stem bark is reddish brown in colour, easily peels off and exudes amber coloured sap. Traditionally, extract of the stem bark is frequently used as antiparasitic and antimicrobial herbs amongst the rural village farmers in Southeast Nigeria. Also, the stem bark extract is commonly used as a preservative to raffia palm (*Raphia farinifera*) wine in certain rural areas of Nigeria particularly parts of Abia, Akwa Ibom and Rivers States of Nigeria (Okoye, 2001), where it is believed to temper the taste, reduce effervescence, impart an amber colour to the wine and preserve it (Nwosu *et al.*, 2001).

The stem bark extract of the plant has also been proved scientifically to decrease the formation of lipid peroxidation products and complement the natural anti-oxidant protection during 2,4-dinitrophenyl hydrazine-induced experimental lipid peroxidation *in vitro* and *in vivo* (Maduka & Okoye, 2002a,b; Nwosu *et al.*, 2010). Other studies have also demonstrated the anti-microbial (Faparusi & Osiyemi, 1973) and anthelmintic (Nwosu *et al.*, 2001) effects of the stem bark extracts of *S. gabonensis*. Owing to the folkloric use of the stem bark of *S. gabonensis* as antiparasitic herbs in most rural poor sub-Saharan African villages, this study aims to assess the toxicity and anti-coccidial effect of aqueous stem bark extract of *S. gabonensis* in broiler chickens experimentally infected with mixed *Eimeria* oocysts.

Material and methods

Experimental animals

They included 119 one day-old, broiler chickens from Obasanjo Farms, Ibadan sales depot. They were housed in a deep litter system at the Dept. of Veterinary Parasitology and Entomology Lab. Animal House, Univ. of Nigeria, Nsukka, and maintained on a standard commercial pelleted poultry feed. Water was provided *ad libitum* and the birds were routinely vaccinated against Newcastle disease and infectious bursal diseases before they were used in the experimental studies.

Preparation of *Sacoglottis gabonensis* extract

The *S. gabonensis* stem bark was collected from *S. gabonensis* trees at Abawsi, Abia State, Nigeria during the months of the rainy season. The stem bark was identified and confirmed at the Dept. of Plant Science and Biotechnology, Univ. of Nigeria, Nsukka. A sample specimen was deposited in the Herbarium Unit. The stem bark was dried, ground into powder and extracted using soxhlet apparatus with water for 8 hours at 60°C. The soluble extract was later concentrated, weighed using a sensitive Mettler® weighing balance and stored at 4°C until required.

Infective material

The intact caeca of chicks known to have suffered and died of natural clinical coccidiosis were dissected out and opened up at post mortem. *Eimeria* oocysts were harvested following standard procedures (Levine, 1973). Briefly, the caeca were carefully cut off from the intestines at the ileocecal junction and contents washed into a beaker, centrifuged and the sediment was mixed with saturated NaCl solution to float the oocysts. Traces of salt and colouring matter were removed by repeated washing with water and centrifugation of the floated oocysts. The harvested oocysts were routinely sporulated using 2.5% potassium dichromate and were identified as a mixture of *Eimeria* species (80% *Eimeria tenella* and 20% *Eimeria maxima*) based on their morphological features and site of isolation. The oocysts were thereafter multiplied in 4 four-week old chicks through oral infection and oocysts recovered from their faecal samples oocysts were sporulated and stored in 2.5% potassium dichromate (Levine, 1973).

To determine the infective dose of the sporulated *Eimeria* oocysts, a total of thirty 4-week old, broiler chickens were used. The birds were divided into six groups (A-F) of five birds each. Groups A-E birds were infected by administering increasing doses of sporulated oocysts: 40,000; 80,000; 120,000; 200,000 and 250,000 sporulated oocysts, respectively, per bird, in a 1 mL aqueous suspensions, using an oral gavage. The birds in group F served as the uninfected control. The birds were assessed daily for clinical signs typical of coccidiosis and their faeces were examined for presence of oocysts. Birds infected with 2×10^5 and 2.5×10^5 sporulated oocysts per birds exhibited the most severe clinical signs of coccidiosis, weight loss, very high oocyst counts and mortality. Thus, 2×10^5 sporulated oocysts per bird was chosen as the infective dose for the study.

Acute and sub-acute toxicity tests

Sixty four-week old birds were used for the toxicity tests. For the acute toxicity test of *S. gabonensis* stem bark

extract (SGSBE), thirty broiler chicks were divided into six groups (A-F) of five birds each. The birds in groups A-E were given graded doses (200, 400, 800, 1,600 and 2,000 mg/kg respectively) of SGSBE orally. Group F (control) birds were given distilled water orally. The chicks were assessed during 24 hours for signs of toxicity and/or death.

For the sub-acute toxicity test, the remaining 30 broiler birds were divided at random into another six groups (A-F) of five birds each. The birds in groups A-E were treated orally with graded concentrations (200, 600, 800, 1,200, and 1,600 mg/kg of SGSBE respectively) of the extract daily for 21 days. Group F (control) birds were also given distilled water orally. Signs of toxicity and mortality were used to assess the sub-acute toxicity of SGSBE.

***In vivo* anti-coccidial effect**

Twenty-five broiler chicks randomly allocated to 5 groups (A-E) of five birds each were used to study the *in vivo* anti-coccidial effects of SGSBE. Each of the birds in groups A-D was orally infected with 2×10^5 sporulated mixed *Eimeria* oocysts while birds in group E served as the uninfected control. On day 4 post infection when oocysts were observed in the faeces of all the infected chicks, groups A and B birds were treated once and for five days orally with 200 mg/kg SGSBE respectively. Group C birds were treated orally with amprolium (100 g/125 L of water) for 5 days whereas birds in group D remained untreated. Following infection, the birds were observed daily for clinical signs of coccidiosis. Oocyst output, body weight, haematological and serum protein parameters of the birds were also determined.

Assessment of parameters

The presence of oocysts in faeces of each bird was determined using the salt flotation technique while oocyst counts per gram of faeces of the individual birds were determined every other day from day 2 post infection till the end of the experiment (day 18 PI) using the modified McMaster technique (Ministry of Agriculture, Fisheries and Food, 1977). Live body weights were determined daily till the end of the experiment (day 18 PI) using a triple beam balance. Red blood cell (RBC) and total leucocyte (TLC) counts were determined using the improved Neubauer technique whereas the microhaematocrit and the cyanomethaemoglobin methods were used to assess the packed cell volume (PCV) and haemoglobin concentration (HbC) respectively (Schalm *et al.*, 1975). Total serum protein (TSP) was assessed using the direct Biuret method (Lubran, 1978) while serum albumin (SA) was determined using the bromocresol green method (Doumas

et al., 1971). The serum globulin concentration (SGC) was estimated by subtracting the SA values from the TSP concentration. The TLC, RBC and PCV of the birds were assessed every other day from the first to the last day of the experiment while the HbC, TSP, SA and SGC were assessed every four days from the first to the last day of the experiment.

Handling of experimental animals

Approval was obtained from the Ethics Committee for Medical and Scientific Research of the University of Nigeria, Nsukka (UNN/FVM/EC/2371). Also, the birds were handled following the stipulated principles guiding the use and care of laboratory animals in biomedical research.

Statistical analysis

Data obtained were analyzed on SPSS 20 for windows using one-way analysis of variance (ANOVA). Least significance difference (LSD) was used to separate variant means and *p* values ≤ 0.05 were considered significant. The survival time of the birds was assessed via Kaplan-Meier survival analysis and log-rank test.

Results

Toxicity tests

The birds that were given single oral doses of 200-800 mg/kg of SGSBE manifested no clinical signs while those that received 1600 and 2000 mg/kg doses of SGSBE were temporarily off feed for about 2 h and 24 h respectively, with 2000 mg/kg dose group recording one mortality during the period. Following prolonged (21 days) oral administration of SGSBE, no sign of toxicity was observed amongst the treated birds except the group that received 1600 mg/kg which were temporarily off feed.

***In vivo* anti-coccidial effect**

Clinical signs and oocyst output

Oocysts were first detected on day 3 post infection (DPI) and by DPI 4 it was present in the faeces of all infected birds reaching peak levels by DPI 8 in group D. Treatments resulted in a significant ($p < 0.05$) reduction in oocysts counts per gram of faeces. Infection was eliminated on days 10, 12 and 15 post treatment in the amprolium and in the five and one day extract-treated groups,

respectively (Fig. 1). The clinical signs observed included depression, ruffled feathers, drooping, bloody diarrhoea with whitish streaks, frank blood in faeces and death. These signs disappeared gradually in all the treated groups whereas it continued in the infected/untreated group D until death of all birds in that group. Two birds survived in the group treated once with SGSBE while all the birds in the 5 days SGSBE-treated group B, amprolium-treated group C and the uninfected control group E survived till the end of the experiment (Fig. 2).

The infected/untreated group of birds recorded a gradual but significant ($p<0.05$) decrease in live weight during the study whereas the uninfected control group showed a continuous increase in weight throughout the study (Fig. 3). Live body weights significantly ($p<0.05$) improved in all the treated groups when compared to the infected/untreated group from DPI 7. Also, the mean live body weights of the extract-treated groups did not vary significantly from the amprolium-treated group.

Haematology and serum proteins

The RBC, PCV and Hbc values of all the infected groups became significantly ($p<0.05$) reduced following infection and remained so until death of all birds in the infected-untreated group D (Tables 1 and 2). However, treatment with amprolium and the extract improved the values of these parameters and were comparable to those of the uninfected control group.

A significant increase ($p<0.05$) was observed in the TLC of the infected groups of birds when compared to the uninfected-untreated group. Following treatment, the

TLC values of the infected groups returned to their normal pre-infection values from days 10 and 12 respectively post infection. The infected-untreated group showed continuous significant ($p<0.05$) increase in TLC values till day 10 PI before it declined beyond the pre-infection values (Table 1).

The treated groups had significantly higher ($p<0.05$) TSP, SA and SGC values compared to the infected/untreated group (Table 2). The values of these parameters were comparable amongst the treated groups except on DPI 16 when the values of SGSBE one day treatment group declined ($p<0.05$) compared to others.

Discussion

The toxicity studies suggest that SGSBE has a relatively wide safety margin when administered orally with LD50 of >1600 mg/kg as only one death was recorded in the group that received the highest dose (1600 mg/kg). This agrees with the views of Hashemi *et al.* (2008) that aqueous plant extracts are generally noxious to birds when given orally at high concentrations. Poor absorption of the toxic components of the extract in the gastrointestinal tract or their degradation by digestive enzymes following oral administration to relatively safer by-products could be responsible for the wide safety margin of the extract.

Classical clinical signs, especially bloody diarrhoea observed in almost all the infected birds were typical of coccidiosis and are akin to the observations of Patra *et al.* (2009). Treatment with SGSBE caused the disappearance of the clinical signs, reduced mortality and significantly improved survivability as 5-days SGSBE-treated birds

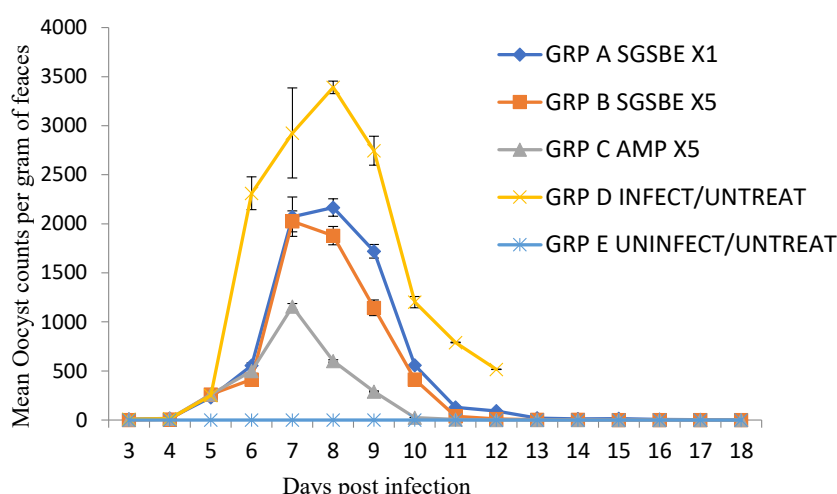


Figure 1. Mean oocyst output of birds infected with mixed *Eimeria* species oocysts and treated with aqueous *Sacoglottis gabonensis* stem bark extract (SGSBE) or amprolium. Bars represent standard error of the mean. Groups A and B were treated once and for five days with 200 mg/kg SGSBE respectively, group C was treated with amprolium (100 g/125 L of water), group D was not treated, while group E was neither infected nor treated.

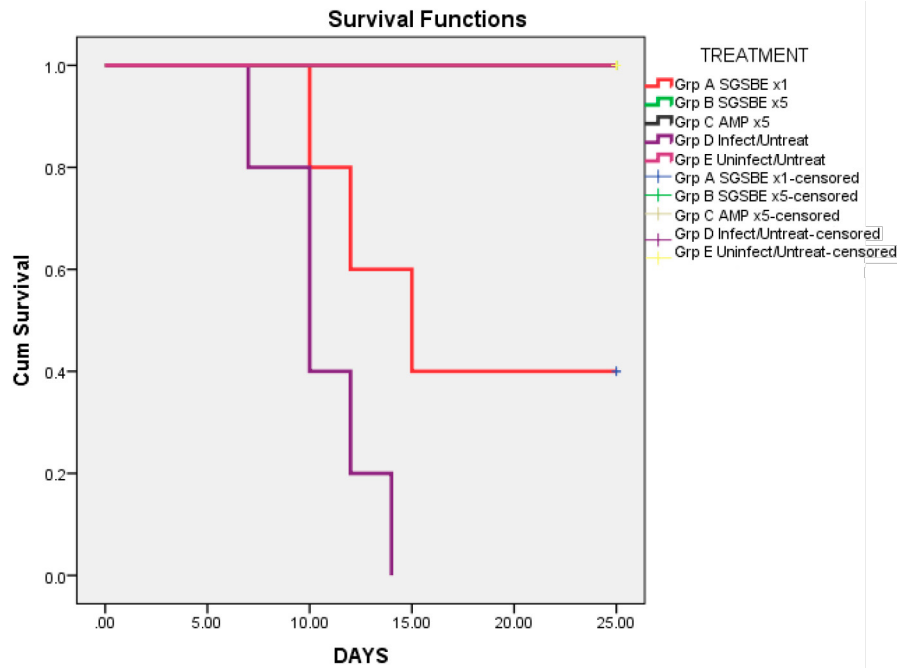


Figure 2. Kaplan-Meier survival estimates of birds infected with mixed *Eimeria* species oocysts and treated with aqueous *Sacoglottis gabonensis* stem bark extract (SGSBE) or amprolium. Groups A and B were treated once and for five days with 200 mg/kg SGSBE respectively, group C was treated with amprolium (100 g/125 L of water), group D was not treated, while group E was neither infected nor treated.

survived beyond the termination of the experiment. These effects could be attributed to the ability of SGSBE in mitigating the effects of the parasite. The antioxidant properties of the extract could also be implicated as antioxidant compounds are known to decrease the severity

of coccidial infections by reducing the extent of intestinal lipid peroxidation and oxidative stress (Allen *et al.*, 1998). Diclazuril, an effective anticoccidial drug is also presumed to produce its result by diminishing the extent of lipid peroxidation (Eraslan *et al.*, 2004; Wang *et al.*,

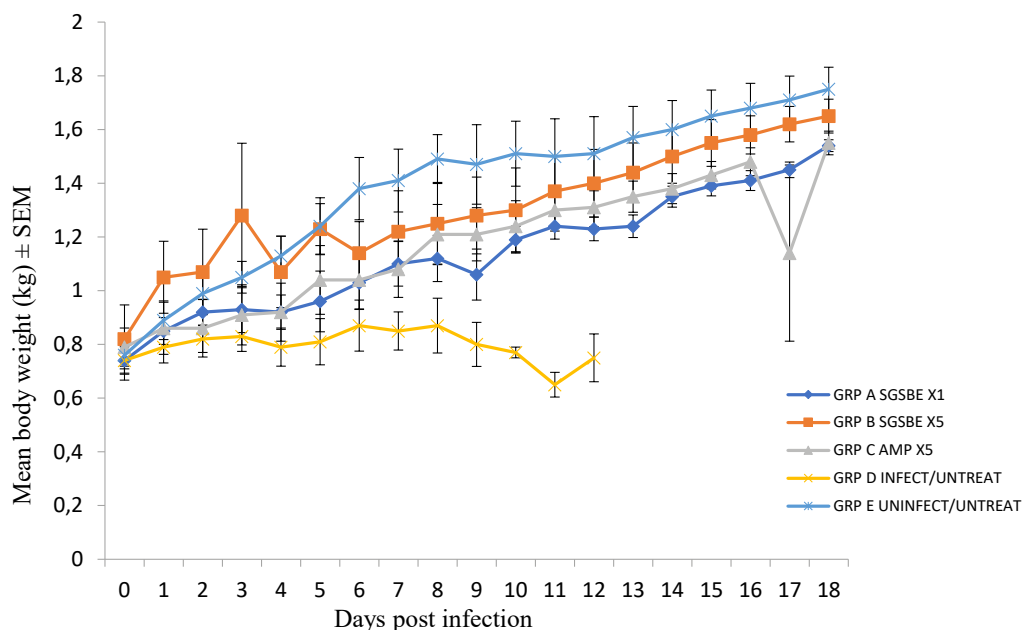


Figure 3. Mean body weight of birds infected with mixed *Eimeria* species oocysts and treated with aqueous *Sacoglottis gabonensis* stem bark extract (SGSBE) or amprolium. Groups A and B were treated once and for five days with 200 mg/kg SGSBE respectively, group C was treated with amprolium (100 g/125 L of water), group D was not treated, while group E was neither infected nor treated.

Table 1. Mean packed cell volume, total leucocyte and red blood cell counts of broiler chickens experimentally infected (groups A-E) with mixed *Eimeria* species oocysts and treated with aqueous *Sacoglottis gabonensis* stem bark extract or amprolium

Days post infection	A	B	C	D	E
Packed cell volume (%)					
0	35.3±0.95	35.5±1.85	35.5±2.25	35.5±0.87	35.3±1.75
2	32.5±1.32	32.3±1.84	31.5±1.04	31.8±1.55	35.5±1.19
4	21.8±1.11 ^a	22.3±1.65 ^a	22.3±2.25 ^a	22.3±1.18 ^a	34.5±0.65 ^b
6	20.3±0.85 ^a	20.3±1.25 ^a	20.3±0.63 ^a	14.5±1.19 ^b	34.3±1.11 ^c
8	20.8±1.38 ^a	21.3±1.11 ^a	27.3±2.56 ^b	14.5±0.87 ^c	34.8±0.75 ^d
10	24.3±2.36 ^a	28±0.71 ^{ab}	31.3±0.48 ^b	13.3±0.33 ^c	35.5±0.96 ^d
12	30.7±1.67 ^a	33.3±1.89 ^a	33±1.35 ^a	12.7±0.33 ^b	34.5±0.5 ^a
14	33±0.58 ^a	34.3±0.85 ^{ab}	35±0.41 ^{ab}		35.3±0.75 ^b
16	34±1 ^a	34.5±0.29 ^{ab}	35.3±0.25 ^{ab}		35.8±0.48 ^b
Red blood cell count (×10⁶/μL)					
0	2.62±0.05	2.53±0.036	2.56±0.02	2.81±0.03	2.56±0.01
2	2.65±0.31 ^a	2.48±0.036 ^{ab}	2.29±0.03 ^{ab}	2.13±0.07 ^b	2.76±0.12
4	2.65±0.03 ^{ab}	2.20±0.013 ^a	2.17±0.01 ^{ab}	2.14±0.02 ^b	2.59±0.02 ^c
6	2.03±0.02 ^a	2.10±0.047 ^a	2.08±0.05 ^a	2.01±0.02 ^a	2.65±0.02 ^b
8	2.08±0.01 ^a	2.21±0.045 ^{ab}	2.30±0.03 ^b	1.47±0.08 ^c	2.62±0.05 ^d
10	2.34±0.01 ^a	2.40±0.006 ^b	2.38±0.02 ^b	1.42±0.00 ^c	2.54±0.01 ^d
12	2.44±0.06 ^a	2.48±0.010 ^a	2.49±0.02 ^a	1.39±0 ^b	2.31±0.24 ^a
14	2.48±0.09 ^{ac}	2.45±0.010 ^a	2.49±0.02 ^{ac}		2.58±0.03 ^c
16	2.53±0.01 ^a	2.56±0.025 ^a	2.5±0.02 ^a		2.55±0.02 ^a
Total leucocyte count (×10³/μL)					
0	5.66±0.30	5.84±0.22	5.64±0.10	5.58±0.20	5.83±0.20
2	10.05±0.16 ^a	10.06±0.34 ^a	10.28±0.18 ^a	10.29±0.28 ^a	6.09±0.12 ^b
4	14.95±0.62 ^a	14.5±0.11 ^a	14.2±0.3 ^a	14.85±0.24 ^a	6.80±0.12 ^b
6	14.73±0.23 ^a	14.75±0.15 ^a	14.45±0.26 ^a	15.05±0.37 ^a	6.54±0.17 ^b
8	14.56±0.49 ^a	14.51±0.30 ^a	11.46±0.34 ^b	15.38±0.39 ^a	5.96±0.25 ^c
10	13.43±1.65 ^a	9.43±0.46 ^b	8.05±0.22 ^{bd}	17.433±0.73 ^c	5.79±0.100 ^d
12	9.12±0.53 ^a	7.9±0.21 ^b	6.26±0.07 ^c	4.10±0.00 ^d	6.33±0.12 ^c
14	9.47±0.15 ^a	8.44±0.31 ^a	6.09±0.18 ^a		6.31±0.18 ^a
16	7.45±0.10 ^a	7.68±0.25 ^a	7.41±0.05 ^a		7.40±0.13 ^a

^{abcd} Different superscripts in a row for each of the hematological parameters represent significant differences between groups at the probability of $p < 0.05$. Groups A and B were treated once and for five days with 200 mg/kg SGSBE respectively, group C was treated with amprolium (100 g/125 L of water), group D was not treated, while group E was neither infected nor treated

2016). Similar findings were reported following studies using *Moringa oleifera* acetone leaf extract, *Artemisia vestita* and methanolic extract of *Punica granatum*, respectively on *Eimeria* species (Ola-Fadunsin & Ademola, 2013; Ahad *et al.*, 2017; 2018).

The faecal oocyst count is consistently used to assess anticoccidial activities of drugs and botanicals (Zhang *et al.*, 2012). SGSBE gradually reduced the faecal oocyst output of the birds reaching zero levels on DPIs 12 and 15 for the five days and one day treatment groups respectively, which is comparable to that of the amprolium treated group. The mechanism of oocyst reduction by the

extract is not completely understood, but it is believed that SGSBE might have some bioactive compounds which either interfered with the multiplication of the parasites or caused the death of the parasites, in addition to the antioxidant properties of the extract. Plant extracts usually have many bioactive compounds and produce their results via the additive or synergistic actions of numerous chemical compounds acting at one or multiple target sites (Tyler, 1999). Phytochemical constituents of SGSBE were found to consist of flavonoids, alkaloids, saponins, tannins, glycosides, terpenoids and phenolic compounds such as bergenin and gallic acid (Ejikeme *et al.*, 2014; Tchouya *et al.*,

Table 2. Mean hemoglobin concentration, total protein and serum albumin levels of broiler chickens experimentally infected with mixed *Eimeria* species oocysts and treated with aqueous *Sacoglottis gabonensis* stem bark extract or amprolium

Groups/Treatment	Days post infection				
	0	4	8	12	16
Hemoglobin concentration (g/dL)					
A	11.4±1.50	9.9±3.50	9.6±0.70 ^a	10.1±1.10 ^a	10.8±0.80
B	11.7±1.20	10.3±1.80	9.5±1.60 ^a	10.2±1.70 ^a	11±0.30
C	11.4±1.10	10±0.80	9.6±0.70 ^a	10.4±1.10 ^a	11±0.50
D	11.6±1.10	10±0.90	6.8±1.10 ^b	6.3±0.60 ^b	
E	11.4±1.50	11.2±1.20	10.8±1.10 ^a	11.1±0.70 ^a	11.4±0.50
Total protein (g/dL)					
A	3.26±0.15	2.78±0.17	2.29±0.09 ^{ab}	2.37±0.11 ^a	2.33±0.18 ^a
B	3.31±0.37	2.69±0.12	2.42±0.11 ^a	2.46±0.11 ^a	2.73±0.08 ^a
C	3.36±0.24	2.64±0.11	2.37±0.11 ^a	2.68±0.19 ^a	2.64±0.04 ^b
D	3.27±0.20	2.64±0.36	2.02±0.09 ^b	1.79±0.00 ^b	
E	3.31±0.11	3.14±0.28	2.73±0.08 ^c	3.04±0.07 ^c	3.09±0.05 ^c
Serum albumin (g/dL)					
A	0.79±0.10	1.09±0.23	0.94±0.04 ^a	0.73±0.07 ^{ab}	0.94±0.00 ^a
B	0.83±0.09	0.85±0.04	1.12±0.22 ^a	0.72±0.07 ^{ab}	1.00±0.02 ^{ab}
C	0.85±0.06	0.84±0.03	0.96±0.06 ^a	0.74±0.05 ^{ab}	0.97±0.04 ^a
D	0.82±0.12	0.78±0.02	0.75±0.03 ^b	0.59±0.00 ^a	
E	0.81±0.10	0.94±0.02	1.23±0.19 ^a	0.82±0.02 ^b	1.06±0.02 ^b
Serum globulin (g/dL)					
A	2.47±0.10	1.69±0.34	1.35±0.09	1.64±0.17	1.39±0.18
B	2.49±0.32	1.84±0.11	1.30±0.22	1.74±0.05	1.73±0.09
C	2.51±0.26	1.80±0.13	1.42±0.09	1.95±0.15	1.67±0.06
D	2.45±0.22	1.86±0.37	1.27±0.11	1.20±0.00	
E	2.5±0.20	2.20±0.28	1.50±0.16	2.22±0.06	2.03±0.03

^{abcd} Different superscripts in a row for each of the hematological parameters represent significant differences between groups at the probability of $p < 0.05$. Groups A and B were treated once and for five days with 200 mg/kg SGSBE respectively, group C was treated with amprolium (100 g/125 L of water), group D was not treated, while group E was neither infected nor treated.

2016). Saponin is believed to induce death of coccidial organisms by binding to membrane cholesterol, changing the parasite membrane resulting in loss of homeostasis (Wang *et al.*, 1998). Reductions in faecal oocyst output have also been reported using different extracts of *Punica granatum*, *Artemisia vestita*, *Moringa oleifera*, garlic and others (Ola-Fadunsin & Ademola, 2013; Dar *et al.*, 2014; Ahad *et al.*, 2017; 2018).

The body weights of the extract treated groups were significantly better than the infected and untreated group. This could either be attributed to the significant reduction in the oocyst count of the birds or the inhibition of the intestinal mucosa inflammation by the extract resulting in an elevated nutrient absorption across the intestinal wall and increased feed conversion ratio, compared to untreated infected birds. It is also likely that SGSBE and amprolium kindle appetite, so the treated birds ate more and improved in weight gain better than those not treated. The findings of the present study are akin to the findings

of Chandrasekaran *et al.* (2009), who reported that herbal complexes consisting of extracts of *Solanum nigrum*, *Moringa indica*, *Aloe vera* and *Mentha arvensis* improved body weight, reduced mortality, faecal oocyst output and lesion scores of treated birds. In the present study, treatment of the infected birds with the extract for 5 days produced significantly better results than the single treatment probably due to the sustained and increased concentration of the extract in the birds.

Haemato-biochemical (PCV, HbC, RBC, TLC, TSP, SGC and SA) indices were also improved following treatment of the infected birds with SGSBE compared to the infected untreated group which may be attributable to the direct anticoccidial, anti-inflammatory and/or the hemopoietic effect of the extract. SGSBE administration was reported to tremendously increase the packed cell volume and red blood cell counts of rats (Maduka & Okoye, 2000). Improvement of haemato-biochemical parameters of *Eimeria*-infected birds following the administration of plant extracts have been

severally reported (Dar *et al.*, 2014; Gotep *et al.*, 2016). Perhaps, these findings of wide safety margin, significant reduction of oocysts burden, reduced mortality, improved body weight and haemato-biochemical parameters justify the folkloric use of the extracts of *S. gabonensis* stem bark by rural sub-Saharan African village farmers for the treatment of most parasitic infections including coccidiosis.

In summary, this study shows that aqueous extract of *Sacoglottis gabonensis* stem bark is safe and possess *in vivo* anticoccidial effects against mixed *Eimeria* species infections in chickens. The anticoccidial effect of the extract was more pronounced when treatment was repeated for five consecutive days rather than one day only. These observations suggest the possible usefulness of the plant in the routine control of avian coccidiosis in rural sub-Saharan African villages and may serve as a lead for the development of an effective alternative anticoccidial drug.

References

- Agishi G, Luga II, Rabo JS, 2016. Prevalence of coccidiosis and *Eimeria* species in layers and broilers at slaughter houses in Makurdi, Benue State. *Int J Eng Sci* 5(2): 8-11.
- Ahad S, Tanveer S, Nawchoo IA, Malik TA, 2017. Anticoccidial activity of *Artemisia vestita* (Anthemideae, Asteraceae)-a traditional herb growing in the Western Himalayas. *Microb Pathogenesis* 104: 289-295. <https://doi.org/10.1016/j.micpath.2017.01.053>
- Ahad S, Tanveer S, Malik TA, Nawchoo IA, 2018. Anticoccidial activity of fruit peel of *Punica granatum* L. *Microb Pathogenesis* 116: 78-83. <https://doi.org/10.1016/j.micpath.2018.01.015>
- Alhotan RA, Abudabos A, 2019. Anticoccidial and antioxidant effects of plants derived polyphenol in broilers exposed to induced coccidiosis. *Environ Sci Pollut Res* 26(14): 14194-14199. <https://doi.org/10.1007/s11356-019-04615-2>
- Allen PC, Danforth HD, Augustine PC, 1998. Dietary modulation of avian coccidiosis. *Int J Parasitol* 28: 1131-1140. [https://doi.org/10.1016/S0020-7519\(98\)00029-0](https://doi.org/10.1016/S0020-7519(98)00029-0)
- Chandrakesan P, Muralidharan K, Kumar VD, Ponnudurai G, Harikrishnan TJ, Rani KSVN, 2009. Efficacy of a herbal complex against caecal coccidiosis in broiler chickens. *Veterinarski Arhiv* 79: 199-203.
- Chapman HD, Jeffers TK, Williams RB, 2010. Forty years of monensin for the control of coccidiosis in poultry. *Poult Sci* 89(9): 1788-1801. <https://doi.org/10.3382/ps.2010-00931>
- Dar SA, Verma P, Ashfaq M, Zargar AA, Mir IA, 2014. Effect of garlic extract on haematobiochemical changes in *Eimeria tenella* infected broiler chicken. *Natl Acad Sci Lett* 37(4): 311-316. <https://doi.org/10.1007/s40009-014-0237-4>
- Doumas BT, Watson WA, Biggs HG, 1971. Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica Chimica Acta* 31: 87-96. [https://doi.org/10.1016/0009-8981\(71\)90365-2](https://doi.org/10.1016/0009-8981(71)90365-2)
- Ejikeme CM, Ezeonu CS, Eboatu AN, 2014. Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta area of Nigeria. *Eur Sci J* 10(18): 247-270.
- Eraslan G, Cam Y, Eren M, Liman BC, 2004. Changes in malondialdehyde level and catalase activity and effect of toltrazuril on these parameters in chicks infected with *Eimeria tenella*. *Bull Vet Inst Pulawy* 48: 251-254.
- Faparusi SI, Osiyemi FO, 1973. The biological effects of bark of *Sacoglottis gabonensis* urban (Humiriaceae) on microorganisms. *Experimenta* 29: 634-635. <https://doi.org/10.1007/BF01926714>
- Gotep JG, Tanko JT, Forcados GE, Muraina IA, Ozele N, Dogonyaro BB, *et al.*, 2016. Therapeutic and safety evaluation of combined aqueous extracts of *Azadirachta indica* and *Khaya senegalensis* in chickens experimentally infected with *Eimeria* Oocysts. *J Parasitol Res* 2016: Art ID 4692424.
- Hashemi SR, Zulkifli I, Hair-Bejo M, Farida A, Somchit MN, 2008. Acute toxicity study and phytochemical screening of selected herbal aqueous extract in broiler chickens. *Int J Pharmacol* 4: 352-360. <https://doi.org/10.3923/ijp.2008.352.360>
- Jatau ID, Sulaiman NH, Musa IW, Lawal AI, Okubanjo OO, Isah I, Magaji Y, 2012. Prevalence of coccidia infection and preponderance *Eimeria* species in free range indigenous and intensively managed exotic chickens during hot-wet season, in Zaria, Nigeria. *Asian J Poult Sci* 6: 79-88. <https://doi.org/10.3923/ajpsaj.2012.79.88>
- Levine ND, 1973. Protozoan parasites of domestic animals and of man, 2nd ed., Burgess, Minn, USA.
- Lubran MM, 1978. The measurement of total serum proteins by the Biuret method. *Ann Clin Lab Sci* 8: 106-110.
- Maduka HCC, Okoye ZSC, 2000. Bergenin, a Nigerian alcoholic beverage additive from *Sacoglottis gabonensis*: as an antioxidant protection of mammalian erythrocytes against lysis by peroxy radicals. *J Med Lab Sci* 9: 88-92.
- Maduka HCC, Okoye ZSC, 2002a. The effect of *Sacoglottis gabonensis* stem bark extract and Bergenin isolate, a Nigerian alcoholic additives on the natural antioxidant defence during 2,4-dinitrophenyl hydrazine induced membrane peroxidation *in vivo*. *Vasc Pharmacol* 39: 21-31. [https://doi.org/10.1016/S1537-1891\(02\)00281-1](https://doi.org/10.1016/S1537-1891(02)00281-1)
- Maduka HCC, Okoye ZSC, 2002b. The antioxidant effect of *Sacoglottis gabonensis* stem bark extract and

- Bergenin isolate, Nigerian alcoholic additives on the peroxidation deterioration of stored vegetable oils. *Pakist J Biol Sci* 5: 585-588. <https://doi.org/10.3923/pjbs.2002.585.588>
- Mbaya AW, Nwosu CO, Onyeyili PA, 2007. Toxicity and anti-trypanosomal effects of ethanolic extract of *Butyrospermum paradoxum* (Sapotaceae) stem bark in rats infected with *Trypanosoma brucei* and *Trypanosoma congolense*. *J Ethnopharmacol* 111: 526-530. <https://doi.org/10.1016/j.jep.2006.12.020>
- Ministry of Agriculture, Fisheries and Food, 1977. Manual of veterinary parasitology laboratory techniques. Tech Bull No. 18. London.
- Muthamilselvan T, Kuo TF, Wu YC, Yang WC, 2016. Herbal remedies for coccidiosis control: A review of plants, compounds, and anticoccidial actions. *Evidence-Based Complement Altern Med* 2016: Art ID 2657981. <https://doi.org/10.1155/2016/2657981>
- Noack S, Chapman HD, Selzer PM, 2019. Anticoccidial drugs of the livestock industry. *Parasitol Res* 118: 2009-2026. <https://doi.org/10.1007/s00436-019-06343-5>
- Nwosu CO, Haruna NK, Ogugbuaja VO, Akinniyi AO, Onyeyili PA, 2001. Anthelmintic efficacy of ethanolic stem bark extracts of *Sacoglottis gabonensis* (Baill.) against strongyline nematodes of small ruminants. *Life Environ Sci* 2: 127-130.
- Nwosu CO, Mobee KM, Gulani IG, Igbokwe IO, Ogugbuaja VO, 2004. Anthelmintic efficacy of aqueous extracts of *Garcinia kola* seed and stem bark against strongylid nematodes of small ruminants. *Niger J Parasitol* 25: 1-5. <https://doi.org/10.4314/njpar.v25i1.37701>
- Nwosu CO, Eneme TA, Onyeyili PA, Ogugbuaja VO, 2008. Anthelmintic efficacy of crude aqueous extract of the stem barks of *Sacoglottis gabonensis* (Baill.). *Fitoterapia* 79: 101-105. <https://doi.org/10.1016/j.fitote.2007.07.010>
- Nwosu CO, Maduka HCC, Mahe A, Adamu U, Nwagbara ND, 2010. Reduction of the effects on the haematology of rats infected with *Trypanosoma congolense* by ethanolic extract of *Sacoglottis gabonensis* stem bark. *Niger J Bot* 23: 41-54.
- Okoye ZSC, 2001. Biological activity of *Sacoglottis gabonensis* stem bark extract, a palm wine additive. *J Biokemistri* 11(2): 79-93.
- Ola-Fadunsin SD, Ademola IO, 2013. Direct effects of *Moringa oleifera* Lam (Moringaceae) acetone leaf extract on broiler chickens naturally infected with *Eimeria* species. *Trop Anim Health Prod* 45 (6): 1423-1428. <https://doi.org/10.1007/s11250-013-0380-9>
- Patra G, Rajkhowa TK, Ali MA, Tiwary JG, Sailo L, 2009. Studies on clinical, gross, histopathological and biochemical parameters in broiler birds suffered from *Eimeria necatrix* infection in Aizawl District of Mizoram, India. *Int J Poult Sci* 8(11): 1104-1106. <https://doi.org/10.3923/ijps.2009.1104.1106>
- Schalm OW, Jain NC, Carro LEJ, 1975. Veterinary hematology, 3rd ed. Lea & Febiger, Philadelphia.
- Tchouya GRF, Obiang GDN, Bongui J, Lebibi J, 2016. Phytochemical study of *Sacoglottis gabonensis* (Baill.) Urb. isolation of bioactive compounds from the stem bark. *Am Chem Sci J* 11(4): 1-5. <https://doi.org/10.9734/ACSJ/2016/22314>
- Tyler VE, 1999. Phytomedicines: back to the future. *J Nat Prod* 62: 1589. <https://doi.org/10.1021/np9904049>
- Usman JG, Gadzama UN, Kwaghe AV, Madziga HA, 2011. Anticoccidial resistance in poultry: A review. *NY Sci J* 4(8): 102-109.
- Wang D, Zhou L, Li W, Zhou H, Hou G, 2016. Anticoccidial effect of *Piper sarmentosum* extracts in experimental coccidiosis in broiler chickens. *Trop Anim Health Prod* 48(5): 1071-1078. <https://doi.org/10.1007/s11250-016-1034-5>
- Wang Y, McAllister TA, Newbold CJ, Rode LM, Cheeke PR, Cheng KJ, 1998. Effects of *Yucca schidigera* extract on fermentation and degradation of steroidal saponins in the rumen simulation technique (RUSITEC). *Anim Feed Sci Technol* 74(2): 143-153. [https://doi.org/10.1016/S0377-8401\(98\)00137-0](https://doi.org/10.1016/S0377-8401(98)00137-0)
- Zhang DF, Sun BB, Yue YY, Zhou QJ, Du AF, 2012. Anticoccidial activity of traditional Chinese herbal *Dichroa febrifuga* Lour extract against *Eimeria tenella* infection in chickens. *Parasitol Res* 111: 2229-2233. <https://doi.org/10.1007/s00436-012-3071-y>