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Anacardium occidentale (LINN) Stem Bark Extracts: Effects on Poultry Colibacillosis Disease

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Abstract

The challenges posed by microbes in poultry industries and the continued search for natural means of combating them inspired this study. The effect of the stem bark extracts of *Anacardium occidentale* on avian colibacillosis was investigated. Ethanol and aqueous extracts of the stem bark of *A. occidentale* were prepared using standard methods. *In vitro* antimicrobial activities of the extracts were evaluated against *E. coli*. Nine groups of 10 broilers (day old chicks; 48 g – 50 g) each were created and bred for a period of eight weeks. Groups 1 to 3 served as the control, while group 4 to 9 served as the test groups. Meat quality, biochemical and haematological assessments were done using standard methods. The maximum zone of inhibition observed was 13.0±0.4 mm at 100 % concentration of the ethanol extract. Both extracts were observed to have a bactericidal / bacteriostatic ratio of 2. The extracts improved the meat quality, blood protein, liver enzymes and renal functions of the broilers compared to the negative control. Again, packed cell volume, haemoglobin and red blood cell counts were increased by the extracts compared to the negative control. On the whole, the results obtained for the extracts were not significantly different ($p > 0.05$) from that of the commercial antibiotics (positive control) results. Therefore, we can infer that crude extracts of *A. occidentale* could be used against *Colibacillosis* disease in place of the conventional commercial antibiotics.

Keywords: *Anacardium occidentale*, stem bark, aqueous and ethanol extracts, broiler chicks, avian colibacillosis

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Introduction

In Africa, poultries are mostly undertaken by smallholder farmers (Thangarasu et al., 2016). It involves the rearing of domestic birds mostly for the purpose of both their meat and eggs. Most of the bird species belong to the super-order of *galloanserae* (fowl), and more specifically the order *galliformes* which includes quails, turkeys and chickens (Thangarasu et al., 2016). Diverse disease conditions such as avian colibacillosis, Newcastle disease, avian coccidiosis, fowl cholera, flu, and other infectious diseases like infectious coryza are the major sources of loss in the poultry industry (Quiroz-Castaneda and Dantan-Gonzalez, 2015). High incidence of these diseases is the major constraint to poultry

business even to commercial agriculture, especially in Nigeria (Marizvikuru et al., 2008).

Avian colibacillosis is caused by a strain of *E coli,* called the avian pathogenic *E coli* (APEC). It is a sub pathotype of extraintestinal pathogenic *E. coli* (ExPEC) which is known as a potential zoonotic agent (Johnson et al., 2008; Kabir, 2010). Avian pathogenic *E. coli* coexists in the gut microbiota of healthy chickens with avian faecal *Escherichia coli* (AFEC) (Ewers et al., 2004; Kabir, 2010). This disease results in significant economic losses and welfare concerns worldwide (Ewers et al., 2004).

Antimicrobial agents such as antiviral, antibiotic and anti-coccidiosis drugs are widely used in the poultry industry for the treatment and control of avian colibacillosis (Morley et al., 2005). However, Public health and Veterinary medicine practitioners are raising alarm over the increase in antimicrobial resistance by bacteria (Barton et al., 2003). Antimicrobial resistant strains have led to numerous problems of many forms, such as increase and spread of multiple antibiotics resistant pathogens, for both humans and animals in addition to increasing the economic cost of poultry production (Kamel, 2001). Hence, studies on poultry feed now focus on finding efficient methods that depend on food supplements to enhance the growth rate and increase the immunity of chicks, which enhances the production efficiency of chicks. The use of plants and their extracts as poultry feed supplements has been proposed because of their positive effect on improving the production performance of birds.

Cashew plant is widespread and its pseudo-fruits and nuts are consumed by humans (Behrauan et al., 2012). Many active substances are found in this plant, such as flavonoids, alkaloids, cardiac glycoside, tannins and phenols (Olaleye et al., 2007). The bark has been reported to possess anti-fungi, anti-parasitic, anti-bacterial, antiseptic and anti-inflammatory properties (Behrauan et al., 2012), in addition to minerals such as zinc, iron, calcium, copper, magnesium, phosphorus, manganese, sodium and potassium that has been reported for it (Ifesan et al., 2013). However, there is no information on the local use of cashew in poultry feed or in their drinking water.

Therefore, this study was carried out to comparatively evaluate the efficacy of crude ethanol and aqueous stem bark extract of *Anacardium occidientale* in the treatment of colibacillosis diseases of chicken with the view of understanding its effectiveness as a potential substitute to commercial antibiotics.

Materials and Methods

The stem bark of *A. occidentale* was sourced from Abakaliki, following identification of the plant by a taxonomist (Dr. Nwankwor) in the Department of Applied Biology, Ebonyi State University, Abakaliki. The ethanol extraction was done using Soxhlet apparatus. Fifty grams (50 g) of each powdered sample of *Anacardium occidentale* stem bark was measured using electronic weighing balance, wrapped in Whatman filter paper and inserted in the tube of the soxhlet apparatus. A 250ml of 70% ethanol (Baramati Agro LTD, India) was poured into a round-bottom-flask of the apparatus and was heated at 70 ^{0}C. The extraction was carried out for 10 hours. The remaining ethanol in the extracts was evaporated to obtain crude extract. This was done in vacuum using a rotary evaporator. This process was repeated until a total of 1 kg of each of the samples were extracted.

The aqueous extract was prepared by macerating 400 g of the powdered bark in 500 mL of cold distilled water for 2 days. It was then sieved using a muslin cloth and concentrated at a temperature of 40 ^{0}C. This was repeated until a total of 1 kg of the samples were extracted. On the other hand, the *E. coli* was obtained from the National Agency for Food and Drug Administration and Control (NAFDAC), Agulu, Anambra State, Nigeria, and was reactivated in nutrient broth for 24 hours and further sub

cultured in nutrient broth and used for the experiments.

In vitro studies

The well diffusion method described by the National Committee of Clinical Laboratory Standards (1993) was used to determine antibacterial activity, while the minimal inhibitory concentration (MIC) of both extracts was determined using the dilution method described by Greenwood (1989). Also, minimum bactericidal concentration (MBC) was ascertained by the method described by Kacaniova et al. (2011).

In vivo Studies

The Acute oral toxicity study was carried out using the up and down method of acute toxicity (OECD, 2008).

Ethics and animal welfare

Animal care and procedures were performed with strict adherence to the guidelines of good experimental practices according to the Code of Practice for Housing and Care of Animals Used in Scientific Procedures, in the Faculty of Science, Ebonyi State University, Abakaliki Ebonyi State, Nigeria. The birds were fed with unmedicated starter and grower rations (CP = 23% and 19%; ME = 3,100 and 3,200 kcal/kg, respectively) *ad libitum,* with constant access to fresh water and light. The birds were housed in a disinfected deep litter system with wood shavings and rice husk as bedding material. The litter was changed once a week till week 8 to prevent cake formation. The litter material when found damp was replaced by a new one. All chicks were offered broiler super starter crumbles ration for the first four weeks followed by broilers finisher ration till the end of the experiment. Feed and water were provided *ad-libitum.* However, it should be noted that this manuscript does not contain clinical studies or patient data.

In vivo antibacterial activity of extracts in broilers infected with E. coli

Experimental Design

The experimental design adopted for this study was the complete randomised design (CRD) method (Table 1). Agri-Ted broiler chicks (day-old) totalling 90 in number were purchased from Obasanjo Farms Nigeria Limited, Ota in Ogun State. The chicks were grouped as shown in table1.

Table 1: The different experimental groups

Group	Treatment
G_A	Not infected and not treated
G_B	Infected and not treated
G_C	Infected and treated with Commercial Antibiotics
G_D	Infected and treated with 1.5 g/L extract bark extract of *A. occidentale*
G_E	Infected and treated with 3 g/L ethanol bark-extract of *A. occidentale*
G_F	Infected and treated with 6 g/L ethanol bark-extract of *A. occidentale*
G_G	Infected and treated with 1.5 g/L aqueous bark-extract of *A. occidentale*
G_H	Infected and treated with 3 g/L aqueous bark-extract of *A. occidentale*
G_I	Infected and treated with 6 g/L aqueous bark-extract of *A. occidentale*

The chicks were acclimated for 7 days before being randomly distributed in their different cages. On the 8th day, infection commenced and lasted for the next 5 days. The infection process was done through drinking water, until colibacillosis infection signs were obvious which included depression, weakness, inappetence, prostration, coughing, breathing problems (snoring-like), loss of appetite, ruffled feathers and slightly bloody mucus diarrhoea characteristic of *Caecal colibacillosis* were the dominant signs. After 5 days of successful infection, pre-treatment with the extracts commenced and lasted for the next 5 consecutive days. All groups' extract were administered by mixing with drinking water**.** After 5 days of pre-treatment with extracts, the birds were observed for another 39 days as they grew, allowing them access to feed and water. At the end of the 56 days experimental period, eight broilers were randomly selected from each group and used for further analysis..

Meat Quality Analysis

At the end of the experiment, eight broilers per treatment (72 broilers in all) were randomly selected and starved of feed for 10 hours. They were sacrificed by cutting the jugular vein to allow proper bleeding and were also used for the meat quality analysis following the methods described by Oko et al., (2016A), Oko et al., (2016B), Oko et al. (2013); Oko et al. (2012).

Blood components analysis

At the end of 56 days of the experiment blood samples were collected from eight chickens per group and used for both biochemical and haematological analysis. The Biuret method with kits described by Dawnay et al. (1991) was used to test for total protein (TP). More so, Albumin (Ab) and Haemoglobin concentrations were analysed as described by Peters et al. (1982). Furthermore, Globulin (Gb) concentration was calculated as the difference between total protein and albumin concentrations as described by Peters et al. (1982), while the haematological components (PCV, haemoglobin and WBC) were by the method of Oshiro et al. (1982). The RBC indices were determined using methods described by Sarma (1990)

Statistical analysis

The data collected was analysed using R statistical software version 4.0.1. Differences in experimental data were established by One-way ANOVA at $p \leq 0.05$ confidence interval. Mean separation was done using the least significant difference (LSD) test. $P < 0.05$ was considered a significant level.

Results

In vitro activities

In Table 1 shown below, no *E. coli* inhibition was observed at 12.5 % concentration for both ethanol and aqueous extracts. The aqueous extract inhibited the *E. coli* only at 100 % concentration of the extract with a zone of inhibition of 7.0±0.4 mm, whereas ethanol extract inhibited *E. coli* at both 50 % and 100 % extract concentration with zones of inhibition of 10.7±0.1 mm and 7.0±0.4 mm respectively. The zone of inhibition on *E. coli* by 100 % concentration of ethanol extract was not significantly different from the zone of inhibition observed for the oxytetracycline.

Table 2: Zones of *E. coli* growth inhibition at different concentrations of the extracts

Conc. (mg/mL)	Extract	Zone of inhibition (mm)
12.5	Aqueous	NI
	Ethanol	NI

25	Aqueous Ethanol	NI NI
50	Aqueous Ethanol	NI 10.7±0.1[a]
100	Aqueous Ethanol	7.0±0.4[b] 13.0±0.4[a*]
0.025	Oxytetracycline	14.0±0.4

Note: NI= No Inhibition. Values are presented as mean ± SD. Different letters indicate significant difference ($p < 0.05$) within a specific concentration of extract, where a>b. * indicate no significant difference ($p > 0.05$) with oxytetracycline.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) of the extracts

Results shown in Table 3 shows that both aqueous and ethanol bark extracts of *A. occidentale* have potent antimicrobial activities on the test microorganism. The aqueous extract gave a bacteriostatic activity of 25 mg/mL and a bactericidal activity of 50 mg/mL, while the ethanol extract gave a bacteriostatic concentration of 50 mg/mL and a bactericidal concentration of 100 mg/mL. nevertheless, both extracts had a minimum bactericidal concentration to minimum inhibitory concentration ratio (MBC/MIC) of 2.

Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal Concentration (MBC) of the extracts against the test pathogens

Extracts	Isolate	MBC (mg/mL)	MIC (mg/mL)	MBC/MIC Ratio
Aqueous	*E.Coli*	50	25	2
Ethanol	*E.Coli*	100	50	2

Clinical signs and mortality rate

Seven days after the post infection stage, some clinical signs were expressed which suggested colibacillosis infection in all infected groups. depression, weakness, in-appetence, prostration, coughing, breathing problems (snoring-like), loss of appetite, ruffled feathers and slightly bloody mucus diarrhea characteristic of C colibacillosis were the dominant signs. No clinical signs were registered in chickens of the non-infected control group. Mortality was registered only in infected non-treated control groups (30%).

Effect of extracts on the meat quality of the broilers

Infection of the broilers with *E. coli* resorted in a significant ($p<0.05$) increase in the drip loss and cooking loss values of the broilers (Figure 1). Pre-treatment of the broilers with the extracts led to restoration of the drip loss and cooking loss values to values that were not significantly different ($p\geq0.05$) when compared to the group pre-treated with oxytetracycline (positive control). There were no significant differences

(p≥0.05) in the drip loss and cooking loss of the group pre-treated with ethanol extract, compared to the group pre-treated with aqueous extract.

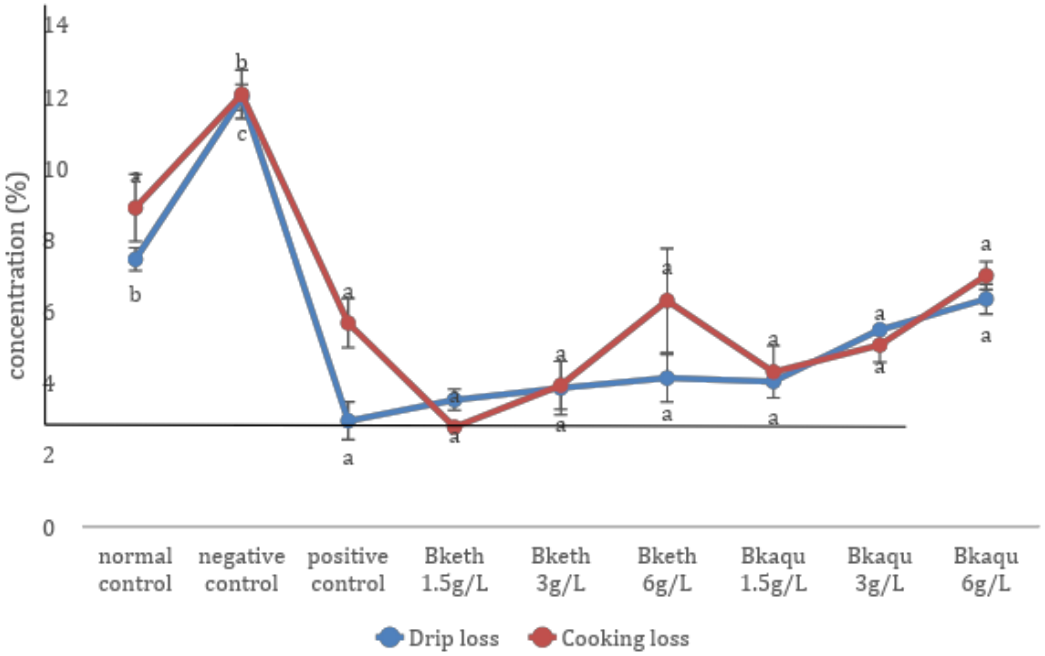

Figure 1: Effect of the extracts on drip loss and cooking loss of the broiler.
Values are presented as mean±SD. Different letters indicate significant difference ($p < 0.05$) across groups where a<b<c. Bketh means Bark ethanol extract, and Bkaqu mean Bark aqueous extract.

Effect of the extracts on haematological/biochemical parameters components of the broilers

The result showed that infecting the broilers with *E. coli* led to a significant ($p<0.05$) decrease in the serum total protein and albumin values of the broiler compared to the normal control group. (Table 4). Pre-treatment with the extracts led to a significant ($p<0.05$) increase in serum total protein and albumin levels of the broilers compared to the negative control groups. The total protein, albumin and globulin values obtained in the groups pre-treated with the extracts were not significantly different ($p < 0.05$) from the values obtained in the positive control group.

Similarly, infection of the broilers with *E. coli* resorted in a significant ($p<0.05$) increase in the alanine transaminase (AST), Aspartate aminotransferase (ALT), alkaline phosphatase (ALP), and cholesterol values of the broilers compared to the normal control (Table 4). Nevertheless, pre-treatment of the broilers with the extract led to a significant ($p<0.05$) decrease in the AST, ALT, ALP and cholesterol levels compared to the negative control group. Serum ALT, ALP, AST and cholesterol levels decreased as the concentration of the extracts increased from 1.5 g/L to 6 g/L.

Table 4: Biochemical parameters of the broilers

Groups	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)	ALT (g/L)	ALP (g/L)	AST (g/L)	Cholesterol (mg/L)

normal control	3.85±0.1^{b}	2.61±0.09^{a}	1.23±0.0^{b}	26.21±1.7^{c}	297.5±0.71^{b}	269±2.83^{b}	105.13±0.14^{c}
negative control	3.27±0.1^{c}	1.99±0.03^{b}	1.86±0.07^{a}	39.84±1.15^{a}	571.5±0.71^{a}	297.5±2.12^{a}	171.27±0.25^{a}
positive control	4.03±0.02^{ab}	2.59±0.13^{a}	1.45±0.1^{b}	33.64±0.71^{bc}	263±2.83^{d}	218.5±2.12^{b}	125.54±0.1^{bc}
Bketh 1.5g/L	3.87±0.13^{b}	2.31±0.02^{a}	1.56±0.15^{a} ^{b}	37.85±1.61^{ab}	286.5±0.71^{c}	208.5±0.71^{cb}	126.13±0.57^{bc}
Bketh 3g/L	4.35±0.21^{a}	2.66±0.11^{a}	1.19±0.32^{b}	35.44±0.62^{bc}	271.5±2.12^{c}	219±2.83^{b}	124.85±0.33^{bc}
Bketh 6g/L	4.24±0.03^{ab}	2.74±0.04^{a}	1.11±0.01^{b}	31.73±1.48^{bc}	265.5±3.54^{d}	225±4.24^{b}	116.13±0.12^{c}
Bkaqu 1.5g/L	3.48±0.23^{bc}	2.56±0.08^{a}	1.29±0.15^{b}	36.87±0.64^{ab}	286±1.41^{c}	174±1.41^{c}	136.91±0.11^{b}
Bkaqu 3g/L	3.89±0.11^{ab}	2.51±0.04^{a}	1.34±0.08^{b}	32.88±0.61^{bc}	265±2.83^{d}	194±7.07^{cb}	133.9±0.22^{b}
Bkaqu 6g/L	4.22±0.06^{ab}	2.64±0.07^{a}	1.21±0.13^{b}	30.73±0.77^{bc}	248±2.83^{d}	195±1.41^{cb}	119.13±0.13^{bc}

Values are presented as mean±SD. Different letters indicate significant difference ($p < 0.05$) across groups where a<b<c. Bketh means Bark ethanol extract, and Bkaqu mean Bark aqueous extract.

Table 5 shows the outcome of the renal function analysis. The infection of the broilers with *E. coli* resorted in a significant ($p<0.05$) in the creatinine level of the broiler compared to the normal control, whereas no significant difference ($p\geq0.05$) was noticed in uric acid and bilirubin values of the negative control group compared to the normal control. The creatinine values of the groups pre-treated with the extracts were significantly ($p<0.05$) lower compared to the negative control group., whereas, there were no significant differences ($p\geq0.05$) in the uric acid and bilirubin levels of the groups pre-treated with the extracts compared to the negative and the positive control groups.

Table 5: Effects of the extracts on the renal function of the broilers

Groups	Creatinine (mg/dL)	Uric acid (mg/dL)	Bilirubin (mg/dL)
normal control	0.825±0.09^{b}	1.37±0.04^{b}	0.24±0.01^{a}
negative control	1.13±0.03^{a}	1.11±0.08^{b}	0.225±0.01^{a}
positive control	0.68±0.01^{b}	2.03±0.02^{a}	0.315±0.05^{a}
Bketh 1.5g/L	0.67±0.06^{b}	1.23±0.02^{b}	0.265±0.02^{a}
Bketh 3g/L	0.8±0.01^{b}	1.31±0.03^{b}	0.245±0.01^{a}
Bketh 6g/L	0.82±0.01^{b}	1.72±0.02^{ab}	0.225±0.01^{a}
Bkaqu 1.5g/L	0.83±0.01^{b}	1.93±0.06^{ab}	0.165±0.02^{a}
Bkaqu 3g/L	0.93±0.08^{b}	2.02±0.01^{a}	0.145±0.02^{a}
Bkaqu 6g/L	1.04±0.01^{b}	2.02±0.02^{a}	0.13±0.03^{a}

Values are presented as mean±SD. Different letters indicate significant differences ($p < 0.05$) across groups where a<b<c. Bketh means Bark ethanol extract, and Bkaqu means Bark aqueous extract.

The haematological results are well elucidated in Table 6 below. The results indicate that infection of the broilers with *E. coli* results in a significant ($p<0.05$) decrease in RBC count of the broilers compared to the normal control group. Pre-treatment of the broilers with the extracts

led to significant ($p<0.05$) increase in the RBC counts of the broilers compared to the negative control. Nevertheless, infection of the broilers with *E. coli* (negative control) had no significant ($p\geq0.05$) effect on the packed cell volume (PCV), haemoglobin, mean corpuscular volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), and WBC count of the broilers compared to the normal control. Pre-treatment of the broilers with the extracts resorted in a subsequent reduction in the MCV and MCH values, and increase in MCHC values, but these values were not significantly different ($p \geq 0.05$) from the values recorded in the positive and normal control groups.

Table 6: Effect of the extracts on the haematological parameters of the broilers

Groups	PCV (%)	Haemoglobin (g/dl)	RBC ($x10^6$/µl)	WBC ($x10^3$/µl)	MCV (fL)	MCH (pg/cell)	MCHC (%)
Normal control	20.56±0.63^{bc}	7.5±0.14^{c}	2.325±0.04^{d}	4.465±0.63^{ab}	88.441^{ab}	32.266^{ab}	36.497^{b}
Negative control	18.96±1.07^{c}	6.43±0.25^{c}	1.805±0.25^{e}	5.24±0.08^{a}	105.626^{a}	36.059^{ab}	34.006^{b}
Positive control	31.65±1.07^{a}	11.88±0.09^{a}	4.32±0.25^{a}	4.345±0.25^{ab}	73.318^{b}	27.541^{b}	37.552^{b}
Bketh 1.5g/L	28.69±0.74^{ab}	9.9±0.56^{b}	2.47±0.21^{c}	3.35±0.21^{bc}	116.456^{a}	40.328^{ab}	34.543^{b}
Bketh 3g/L	28.29±1.56^{ab}	10.49±0.33^{b}	2.755±0.28^{c}	3.24±0.03^{c}	103.557^{a}	38.243^{ab}	37.186^{b}
Bketh 6g/L	31.31±0.79^{a}	9.31±0.12^{b}	3.16±0.01^{bc}	2.85±0.1^{c}	99.089^{a}	29.479^{ab}	29.756^{b}
Bkaqu 1.5g/L	25.54±0.49^{abc}	10.41±0.12^{b}	2.74±0.1^{bc}	4.04±0.02^{b}	93.305^{a}	38.009^{ab}	40.772^{ab}
Bkaqu 3g/L	26.97±0.65^{abc}	10.22±0.11^{b}	3.19±0.01^{bc}	3.88±0.13^{bc}	84.542^{b}	32.037^{ab}	37.900^{b}
Bkaqu 6g/L	28.55±0.57^{ab}	11.745±0.22^{a}	3.475±0.25^{b}	3.18±0.23^{bc}	82.309^{b}	33.907^{ab}	41.154^{ab}

Values are presented as mean±SD. Different letters indicate significant differences ($p < 0.05$) across groups where a<b<c. Bketh means Bark ethanol extract, and Bkaqu means Bark aqueous extract.

Discussion

It is evident that this study clearly demonstrated that both extracts of the stem bark of *A. occidentale* possess potential phytobiotic activities against *E. coli,* the primary cause of avian colibacillosis of the poultry industries (Nolan et al., 2013). This was implied in the results elucidated above. The antimicrobial activities of the bark-extracts of *A. occidentale* were effective against *E. coli* with a MBC/MIC ratio of 2. This observation was in line with the findings of Onuh et al. (2017) who reported that ethanolic extracts of cashew leaves and stem barks showed appreciable inhibition diameter against all bacterial pathogens (*E. coli* inclusive).

Again, stress increases the susceptibility of birds to all diseases (Vandekerchove et al., 2004; Barnes, 2013) and colibacillosis in particular (Johnson et al., 2008). As a matter of fact, stress predisposes birds to opportunistic infections and as such antimicrobials also play

an outstanding role in stress management. Since the extracts of *A. occidentale* showed activities against *E. coli*, a primary cause of avian colibacillosis, it is therefore useful as an antistressor agent in ethno-veterinary medicine (Sarkodie et al., 2015). The activities of these extracts against *E. coli* may be as a result of the synergistic action of some of its constituent phytochemicals such as phenols, flavonoids, alkaloids, and saponins among many others possibly reported for the plant (Aiswarya et al., 2011; Aderiye et al., 2014). According to Aroche et al. (2018) this natural product in cashew has antibacterial effects against strains of *E. coli.*

Again, it is apparent from the drip loss and cooking loss analyses that the extracts improved the meat qualities of the broilers, in addition to being good antibacterial agents. Oko et al. (2012; 2013) reported that the water holding capacity (WHC) of broiler meat is measured as the fraction of bound water retained in the muscle. Therefore, treatment with the plant extracts, which led to reduced drip loss and cooking loss, could be said to have enhanced the water holding capacity of the broiler meat hence, improved the meat quality of the broilers.

In addition, the findings of the present study suggest that the extracts improved the blood protein concentrations of the broilers compared to the negative control. The concentrations of total protein in the serum of the broilers were significantly ($p < 0.05$) lower in the negative control compared to the normal control. This finding is in agreement with the work of Umar et al. (2020) who reported that mean total protein content of *E. coli* infected chicken (5.431 ± 0.042 g/dL) was significantly lower when compared with non-infected chicken (6.154 ± 0.034 g/dL). This decrease in blood proteins may be attributed to severe hepatic damage, found in colibacillosis (Umar et al., 2020).

Similarly, the liver function tests, ALP, ALT and AST lend wait to the activities of the liver in broilers (Ohaeri, 2001). An elevated serum level of these enzymes was observed in the negative control group, indicating liver injury, thus, showing enzymes' spillage from the liver cytosol into the bloodstream (Shah and Khan, 2014). Nevertheless, treatment with the extracts resorted to a significant ($p<0.05$) decrease in the serum concentration of these enzymes. The observed effects of these extracts on the liver enzymes suggests that they can be harnessed for the treatment of avian colibacillosis in the poultry industry.

Furthermore, the extracts were able to restore the serum creatinine, uric acid and bilirubin concentrations to their normal levels. According to Choudhary et al. (2015), creatinine is very important in determining the synthetic and excretory roles of the kidney and liver. They further opined that about 50% of kidney function must be lost before a rise in the serum creatinine can be detected. This is in line with the findings of this study because, infecting the broilers with *E. coli* resorted in significant ($p<0.05$) increase of serum creatinine proving a loss of liver or kidney function, whereas, treatment with the extracts led to a subsequent reduction in the creatinine levels, signalling a restoration of the liver and kidney functions. Also, in support of this assertion, Omotoso et al. (2012) found that the ethanolic extract of *Anacardium occidentale* had a cleansing effect on the kidney.

The results on the effect of extract administration on haematological parameters, RBC, PCV and haemoglobin (Hb) count are in line with the report of Sharma et al. (2016) who observed a decrease in the Hb, PCV and RBC of *E. coli* infected broiler birds. The significant ($p<0.05$) decrease in RBC count of the negative control group may be as a result of breakdown of erythrocytes (RBC) by hemolytic enzymes produced by *E. coli* (Justice et al., 2006), or due to lack of appetite and diarrhoea leading to nutritional deficiencies, which leads to decrease in number of erythrocytes, thus resorting to decrease in per cent PCV and Hb concentration

(Feldman et al., 2000). Nevertheless, pre-treatment of the broilers with the extracts restored the PCV, haemoglobin, and RBC count to their normal ranges in chicken; the normal ranges are - PCV: 22-30 %, Hb: 7-13 g/dl, and RBC: 2.5-3.5 $x10^6$ ul (Bounous and Stedman, 2000).

In addition, average erythrocyte size, haemoglobin amount per blood cell, and the amount of haemoglobin relative to the size of the cell per red blood cells were studied. Although, the MCV, MCH and MCHC values reported in this work were lower than broiler normal means values of 174 fL for MCV, 61pg/cell for MCH and 33 g/dl for MCHC reported by Merck (2012) for broilers, it is worthy of note that these values reported in this study are in line with the MCV, MCH and MCHC values reported for broilers by Onunkwo et al. (2018), Odunitan-Waya et al. (2018), Ifelayo et al. (2020) and Muneer et al. (2021), and within the normal ranges of 90-140fL (MCV), 33-47 pg/cell (MCH) and 26-35 g/dl (MCHC) reported for broilers by Bounous and Stedman (2000). Hence, the ability of the extracts to maintain the MCV, MCH, MCHC levels of colibacillosis infected broilers indicates that they may possess phytobiotic properties against the *E. coli* as well as the tendency to restore blood quality.

Finally, the suggested phytobiotic activities of these extracts could further be explained by the observed effects of the extracts on the WBC count of the birds. The white blood cells are involved in protecting the body against infections. They kill virus-infected cells, and enhance the production of antibodies (Olugbemi et al., 2010). A high concentration of white blood cells in the body connotes a threat to normal health, therefore, the body builds up its defence against such threat (Olugbemi et al., 2010). So, it is apparent from this study that the extracts improved the ability of the bird to fight infections, to defend their bodies against foreign organisms' invasion, and to produce and distribute antibodies. This is because the extracts were able to maintain the white blood cell count of the broilers within the reference values of 1.2-3.0 $x10^3$µl (Jain, 1993), and the normal mean value of 5.5 $x10^3$ µl (Merck, 2012) for broilers.

Conclusion

From the results, it can be concluded that the stem bark extracts of *A. occidentale* are effective against the bacteria studied which could be attributed to the presence of both primary and secondary metabolites contained in the extracts. This further suggests that the extracts could serve as a potential replacement to antibiotics in poultry production for the treatment of avian colibacillosis and other microbial infections. Consequently, further research is recommended in order to ascertain the exact mechanism of action of the extracts.

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