RESPONSE OF LAYING HENS TO AQUEOUS EXTRACTS OF *PETIVERIA ALLIACEA* ROOT AND LEAF

A.M. Oyeleke[1], O.A. Adeyemi[1], L.T. Egbeyale[1], R.A. Sobayo[2], R.O. Olaifa[1]

[1]Federal University of Agriculture Abeokuta, College of Animal Science and Livestock Production, Department of Animal Production and Health, Abeokuta, Nigeria
[2]Federal University of Agriculture Abeokuta, College of Animal Science and Livestock Production, Department of Animal Nutrition, Abeokuta, Nigeria

This study investigated the response of laying hens to aqueous extracts of *Petiveria alliacea* root and leaf. A total of 288 eighteen-week-old Isa brown pullets were used for the 25-week study. The pullets were arranged in a 2 × 4 factorial experimental layout in a completely randomized design. The pullets were distributed into two groups administered root extract or leaf extract. Pullets in each group were allotted to four subgroups administered aqueous extracts of *Petiveria alliacea* at 15, 30 and 45 g l^{-1} concentration levels making eight treatments in total. Each treatment was replicated three times with twelve pullets per replicate. *Eimeria* oocyst counts and intestinal bacteria counts were lower ($P < 0.0001$ and $P = 0.0028$, respectively) in hens administered 15, 30 and 45 g l^{-1} of *Petiveria alliacea* extracts than the control. The highest ($P < 0.0001$) antibody titre against Newcastle disease vaccine was recorded in hens administered 30 and 45 g l^{-1} concentrations of root (9.06 and 9.10 $\log_2$, respectively) and leaf (9.08 and 9.18 $\log_2$, respectively) extracts. The liver sections of hens in all treatments appeared normal. In conclusion, aqueous extract of *Petiveria alliacea* root and leaf at 30 and 45 g l^{-1} concentrations performed best as antimicrobial and immune stimulating agent without impairing liver health.

antibody titre, faecal oocyst count, histopathology of liver, intestinal bacteria count, lymphoid organ

sciendo

doi: 10.2478/sab-2021-0004
Received for publication on December 13, 2020
Accepted for publication on May 4, 2021

INTRODUCTION

For many years there has been a restriction on the use of synthetic drugs such as antibiotics in livestock production due to the emergence of drug-resistant microorganisms and detection of harmful drug residues in animal products (Diarra, Malouin, 2014; Gonzalez, Angeles, 2017). On the other hand, the increasing awareness of the public on food safety has influenced consumer's preference toward food products from organic sources (Biswas et al., 2010). These have necessitated researches aiming to find viable alternatives to synthetic drugs which could serve as growth-promoting, prophylactic and therapeutic agents in poultry production (Diarra, Malouin, 2014). Among these alternatives, medicinal plants have gained significant attention due to their availability, potency and insignificant side effects (Jelveh et al., 2018). Several studies have shown the potentials of various medicinal plants as growth promoters, antimicrobial agents, anticoccidial agents, immunostimulants and hepato-protective agents (Attia et al., 2017a, b; Kilany et al., 2018; Sahoo et al., 2019). However, there is no standardisation yet on the use of these medicinal plants as many studies are still underway and a vast amount of these beneficial plants are yet to be comprehensively assessed.

Petiveria alliacea (Guinea hen weed) is a potent medicinal herb whose medicinal qualities are yet to be explored in the poultry industry. It is a perennial shrub with a rigid and straight stem, the roots, leaves and stems are characterised with garlic odour (Duarte, Lopes, 2005). The plant is native to Northern America, Central America, Southern America and the Caribbean. It was introduced and grown widely in African countries like Nigeria and Benin Republic (Duarte, Lopes, 2005; Schmelzer, Gurib-Fakim, 2008). *Petiveria alliacea* can be found in abundance in the southern part of Nigeria (Alegre, Clavo, 2007). *Petiveria alliacea* is commonly distributed along river banks, road sides, edges of humid forests, pastures and gardens, its seeds can be easily

dispersed by wind or animals favouring colonisation of new areas (Alegre, Clavo, 2007). All parts of the plant were found useful in folk medicine as treatments for various diseases and conditions such as fever, respiratory ailments, nervous spasm, asthma, paralysis and bolstering the immune system (Randle et al., 2018). Researchers have successfully isolated several bioactive compounds from *Petiveria alliacea* most of which are sulphur-containing compounds (dibenzyl-disulfide and dibenzyl-trisulfide), terpenoids, flavonoids, tannin, alkaloids, phytates, phenols, saponins, oxalates, carotenoids and antioxidants (de Andrade et al., 2012; Ekunseitan et al., 2016). Other studies also confirmed the antibiotic, antioxidant, anti-inflammatory, anti-cancerous, anti-carcinogenic, anti-parasitic and immune-stimulating properties of the plant (Kim et al., 2006; Williams et al., 2007; Schmelzer, Gurib-Fakim, 2008; Randle et al., 2018).

In an *in-vitro* study by Ekunseitan et al. (2016) reported the moderate antibacterial activity of *Petiveria alliacea* against selected bacteria of poultry importance. Moreover, few other researchers have investigated the benefits of *Petiveria alliacea* in poultry production (Sobayo et al., 2017, 2018a, b; Muhammad et al., 2019; Odetola et al., 2019). However, these researchers focused their investigations mainly on the use of *Petiveria alliacea* as a growth-promoting agent. The potential benefits of *Petiveria alliacea* as an antibiotic and immune-stimulating agent is yet to be explored in poultry species. Therefore this study investigated the microbial count, immunomodulatory response and liver histopathology in laying hens administered aqueous extracts of *Petiveria alliacea* root and leaf.

MATERIAL AND METHODS

Ethical statement and study site

This study was conducted according to the guidelines of Animal Welfare Committee, College of Animal Science and Livestock Production, Federal University of Agriculture Abeokuta, Nigeria.

The experiment was conducted at the research unit of the Livelihoods Support and Development Centre (SLIDEN AFRICA), Abeokuta, Ogun State, Nigeria. The site is located in the rainforest vegetation zone of south-western Nigeria, latitude 7° 13′29.01″ N and longitude 3° 25′ 26.40″ E, 126.19 m a.s.l., eye altitude 379.48 m (Google Earth, 2019).

Aqueous extraction of *Petiveria alliacea* root and leaf

The extraction was carried out according to the method described by Nodu et al. (2016). Briefly, 15, 30 and 45 g of fresh *Petiveria alliacea* roots and leaves sourced from Kotopo area of Abeokuta, Ogun State were weighed individually. Each weighed root or leaf sample was blended separately in one litre of water using a Panasonic Mx-Gx1021 blender. Each blended mixture was separated by filtration after blending using a 0.1 mm sieve screen. The filtrate from each separated mixture was presented to hens as drinking water according to treatment. In summary, the root and leaf extracts were prepared separately at 15, 30 and 45 g l^{-1} concentrations. Extracts were prepared and stored in a refrigerator prior to days of administration.

Experimental design and management

Two hundred and eighty-eight (288) eighteen-week old Isa brown pullets were used for this study. The experiment, which lasted for 25 weeks, was arranged in a 2 × 4 factorial experimental layout in a completely randomised design. Two factors (plant part and extract concentration) were considered at two (root and leaf) and four (0, 15, 30 and 45 g extract per l) levels, respectively. The birds were distributed into two groups administered root extract or leaf extract. Birds in each group were allotted to four subgroups receiving 0 (control), 15, 30 or 45 g l^{-1} concentration of the extracts. Each treatment was replicated three times with twelve birds per replicate group. The aqueous extracts of *Petiveria alliacea* root and leaf were presented to birds via drinking water on 2 consecutive days per week. On other days of the week, all experimental birds were given ordinary water. The birds were raised in a comfortable battery cage system and fed a mash diet formulated according to the National Research Council (NRC, 1994) recommendation (Table 1). Birds in all treatments did not receive any antibiotics or anticoccidial drug throughout the study.

Data collection and evaluation

Phytochemical analysis of *Petiveria alliacea* root and leaf. Fresh root and leaf samples were air dried at room temperature and pounded to powder. Totally 100 g of powdered root and leaf were put separately into 300 ml of distilled water to soak for 48 h at room temperature. The suspensions were filtered with a Whatman's No. 1 filter paper and the filtrates evaporated to dryness in a water bath at 40°C. Testing for major phytochemical constituents was carried out according to Harborne (1973), Sofowora (1993) and Evans (2000).

Body weight gain. The body weights of experimental birds were determined at the beginning and at the end of the experiment using a Camry digital weighing scale. Average body weight gain per bird was calculated according to the formula:

Average body weight gain per bird = (final body weight of replicate – initial body weight of replicate)/ number of birds in replicate

Table 1. Composition of experimental diet

Ingredients	(%)
Whole maize	48.00
Soya bean meal	11.00
Fish meal (72 % CP)	2.10
Groundnut cake	5.00
Palm kernel cake	8.20
Wheat offal	22.00
Oyster shell	0.50
Bone meal	2.50
Lysine	0.10
Methionine	0.10
Common salt	0.25
Premix	0.25
Total	100.00
Calculated analysis	
Dry matter	89.10
Crude protein	18.25
Crude fibre	5.50
Ether extracts	3.25
Ash	6.50
Metabolisable energy (MJ/kg)	11.67

Premix (content per kg diet): vitamin A 10 000 000 IU, vitamin D_3 2 000 000 IU, 12 500 IU, vitamin K 130 g, vitamin B_2 4 g, D-calcium pantothenate 1.30 g, vitamin B_6 1.30 g, vitamin B12 0.01 g, nicotinic acid 15 g, folic acid 0.05 g, biotin 0.02 g, Co 0.20 g, Cu 5 g, Fe 25 g, I 0.06 g, Mn 48 g, Se 0.10 g, Zn 45 g, chlorine chloride 200 g, BHT 50 g

Faecal *Eimeria* oocyst count. At the end of the experiment, separate trays covered with aluminium foil were used to collect fresh faecal samples from all replicates in each treatment. The fresh faecal samples were picked using sterile forceps into separate labelled sterile bottles. The faecal samples were packed in ice and transported to the laboratory for egg count analysis using McMaster egg counting technique as outlined by Zajac, Conboy (2012).

Intestinal bacteria count, lymphoid organ weight evaluation and serum antibody titre. Fourteen days prior to the end of the experiment, four birds per replicate were selected and housed on a treatment and replicate basis in separate cages. These birds were administered Newcastle disease vaccine (NDV) (LaSota strain) once via drinking water. Routine administration of *Petiveria alliacea* extracts was maintained.

At the end of the experiment, live weights of these four selected birds per replicate were determined using a Camry digital weighing scale. Totally 2 ml of blood was collected aseptically from each hen into labelled plain bottles, and then the birds were slaughtered. Small intestines of the slaughtered hens were carefully isolated from the carcass into separate labelled sterile bottles. The bottles were placed in ice and transported to the laboratory for bacterial count using the viable count method as described by Bassiri (2013). A mixed digesta of each intestinal sample was used to determine the bacterial count. Briefly, 1 g of each digesta was diluted serially (1 : 10) in saline solution to 10^{-6}. An agar plate was then prepared with 0.1 ml of each dilution to determine total bacteria units present. The obtained numbers of colony-forming units (cfu) were expressed as logarithm to base 10.

Lymphoid organs (bursa, thymus and spleen) of the slaughtered hens were isolated from the carcass, weighed individually using a sensitive weighing scale (LEADZM electronic scale) and the weights were expressed as a percentage of live body weight.

For serum antibody titre, the blood samples collected were allowed to clot, the sera were separated into other labelled sterile bottles. A serum antibody titre against NDV was determined by a haemagglutination inhibition (HI) test as described by OIE (2008) using Biovac® vaccine (Biovac, Israel) as antigen. Results obtained from the HI tests were expressed as logarithm to base 2.

Liver histopathology. The liver of slaughtered birds was carefully isolated from the carcass. The liver samples were weighed using a sensitive weighing scale (LEADZM electronic scale) and the weights were expressed as a percentage of live body weight after which they were fixed in 10 % formalin solution and transferred to the laboratory for histopathological study. Histopathological examination was carried out according to the method of Slaoui, Fiette (2011).

Statistical analysis. Data from phytochemical screening of root and leaf were subjected to Student's *t*-test, other data were subjected to Two-way Analysis of Variance in a 2 × 4 factorial arrangement. Significant differences among treatment means were separated using Duncan's Multiple Range Test (Duncan, 1955) as contained in the SAS software package (Statistical Analysis System, Version 9.3, 2010).

Model of the study

$$Y_{ijk} = \mu + T_i + L_j + (TL)_{ij} + \varepsilon_{ijk}$$

where:
Y_{ijk} = output parameter
μ = overall mean
$T_i = i^{th}$ effect of the plant part (i = root, leaf)
$L_j = j^{th}$ effect of the extract concentration (j = 0, 15, 30, 45 g l^{-1})
$(TL)_{ij}$ = interactive effect of the plant part and the extract concentration.
$k = k^{th}$ observation in a treatment (i, j)
ε_{ijk} = residual error

Table 2. Phytochemical analysis of root and leaf of *Petiveria alliacea*

Parameters (mg 100 g^{-1})	Root	Leaf
Saponin	$96.66^{b} \pm 0.88$	$388.66^{a} \pm 0.88$
Alkaloid	$760.00^{a} \pm 0.58$	$549.33^{b} \pm 0.88$
Flavonoid	$263.33^{b} \pm 0.88$	$822.00^{a} \pm 0.58$
Terpenoid	$189.33^{b} \pm 0.88$	$229.00^{a} \pm 0.58$
Tannin	$943.00^{a} \pm 1.15$	$788.67^{b} \pm 1.2$
Anthraquinone	$629.00^{a} \pm 0.58$	$83.00^{b} \pm 1.15$
Carotenoid	$511.67^{b} \pm 0.88$	$648.67^{a} \pm 0.88$
Oxalate	$1\,261.33^{a} \pm 0.88$	$1\,138.00^{b} \pm 0.58$
Phenolics (GAE g^{-1})	$567.67^{a} \pm 0.88$	$423.00^{b} \pm 1.15$

a,bmeans in the same row not sharing common superscript are significantly ($P < 0.05$) different

RESULTS

Concentrations of phytochemicals identified in *Petiveria alliacea* root and leaf are presented in Table 2. Alkaloid, phenolics, tannin, anthraquinone and oxalate contents were higher in the root while saponin, flavonoid, terpenoid and carotenoid concentrations were higher in the leaf.

The effects of various levels of aqueous extract concentrations of *Petiveria alliacea* parts on *Eimeria* faecal oocyst count and intestinal total bacteria count of laying hens are presented in Table 3. *Eimeria* oocyst counts in faeces of hens administered 15, 30 and 45 g l^{-1} of *Petiveria alliacea* root and leaf extracts were significantly ($P < 0.0001$) lower than the values obtained in hens in the control groups. Hens administered 15, 30 and 45 g l^{-1} root extract and hens administered 30 and 45 g l^{-1} leaf extract recorded lower

Table 3. Effect of various levels of aqueous extract concentrations of Petiveria alliacea parts on Eimeria faecal oocyst count and intestinal total bacteria count of laying hens

Treatment		Faecal oocyst count (opg);	Intestinal bacteria count (log_{10} (cfu g^{-1}))
Plant parts	Concentration of extraction (g l^{-1})		
Root		39.75	1.04
Leaf		42.08	1.14
SEM		12.64	0.12
	0	108.33^{a}	1.56^{a}
	15	21.67^{b}	1.10^{b}
	30	18.67^{b}	0.85^{b}
	45	15.00^{b}	0.84^{b}
	SEM	6.34	0.12
Root	0	110.00^{a}	1.57^{a}
	15	20.00^{b}	0.97^{b}
	30	17.33^{b}	0.82^{b}
	45	11.67^{b}	0.80^{b}
Leaf	0	106.67^{a}	1.57^{a}
	15	23.33^{b}	1.24^{ab}
	30	20.00^{b}	0.88^{b}
	45	18.33^{b}	0.87^{b}
SEM		9.58	0.17
***P*-values**			
Plant part		0.7667	0.4585
Extract concentration		< .0001	0.0028
Plant part × Extract concentration		< .0001	0.0240

Table 3. Effect of various levels of aqueous extract concentrations of *Petiveria alliacea* parts on *Eimeria* faecal oocyst count and intestinal total bacteria count of laying hens

SEM = standard error of the mean, opg = oocyst per gram

[a,b]means in the same row not sharing common superscript are significantly ($P < 0.05$) different

Table 4. Effect of various levels of aqueous extract concentration of *Petiveria alliacea* parts on body weight, liver weight, lymphoid organ weights and serum antibody titre of laying hens

Treatment		Initial body weight (g)	Final body weight (g)	Weight gain (g)	Weight of slaughtered hens (g)	Liver (%)	Bursa (%)	Thymus (%)	Spleen (%)	Antibody titre against NDV (log_2)
Plant parts	Extract concentration (g l^{-1})									
Root		1122.72	1832.66	702.35	1825.07	1.32	0.16	0.36	0.10	8.51
Leaf		1117.31	1820.32	698.10	1815.41	1.32	0.16	0.36	0.11	8.53
SEM		13.57	11.47	11.63	9.74	0.05	0.01	0.01	0.01	0.23
	0	1108.43	1807.34	709.40	1817.83	1.25	0.14^{b}	0.32^{b}	0.09^{b}	7.28^{c}
	15	1108.69	1831.13	703.30	1811.99	1.29	0.17^{a}	0.37^{a}	0.11^{a}	8.59^{b}
	30	1107.49	1827.93	716.62	1824.11	1.33	0.16^{a}	0.36^{a}	0.10^{a}	9.07^{a}
	45	1155.45	1839.55	671.59	1827.04	1.39	0.17^{a}	0.39^{a}	0.12^{a}	9.14^{a}
	SEM	16.49	16.25	14.69	13.57	0.07	0.01	0.01	0.01	0.06
Root	0	1095.06	1815.81	719.19	1814.25	1.25	0.14^{bc}	0.32^{b}	0.09^{b}	7.33^{c}
	15	1098.55	1838.27	712.93	1811.48	1.30	0.17^{a}	0.37^{a}	0.11^{a}	8.56^{b}
	30	1138.33	1841.06	705.77	1844.10	1.31	0.16^{ab}	0.36^{a}	0.10^{ab}	9.06^{a}
	45	1158.94	1835.49	671.51	1830.45	1.40	0.18^{a}	0.39^{a}	0.11^{a}	9.10^{a}
Leaf	0	1121.80	1798.88	699.60	1821.40	1.26	0.13^{c}	0.32^{b}	0.09^{b}	7.23^{c}
	15	1118.82	1823.98	693.67	1812.49	1.28	0.17^{a}	0.37^{a}	0.10^{ab}	8.62^{b}
	30	1076.65	1814.80	727.47	1804.12	1.35	0.17^{a}	0.36^{a}	0.10^{ab}	9.08^{a}
	45	1151.96	1843.61	671.67	1823.63	1.38	0.17^{a}	0.38^{a}	0.12^{a}	9.18^{a}
SEM		18.76	23.48	20.73	19.24	0.10	0.01	0.01	0.01	0.09
***P*-values**										
Plant part		0.7851	0.5003	0.8078	0.5361	1.0000	0.8821	0.9224	0.8339	0.8433
Extract concentration		0.2616	0.6342	0.2974	0.8997	0.6414	0.0019	0.0005	0.0041	< .0001
Plant part × Extract concentration		0.4118	0.8952	0.6560	0.9223	0.9615	0.0169	0.0057	0.0323	< .0001

SEM = standard error of the mean, NDV = Newcastle disease vaccine

$^{a–c}$means in the same row not sharing common superscript are significantly ($P < 0.05$) different

values presented for liver, bursa, thymus and spleen were expressed as percentage of the live body weight of slaughtered hens

($P = 0.0240$) intestinal total bacterial counts than the control treatments.

The effects of various levels of aqueous extract concentrations of *Petiveria alliacea* parts on body weight, liver weight, lymphoid organ weights and serum antibody titre of laying hens are presented in Table 4. Hens maintained on 15 and 45 g l^{-1} root extract, 15, 30 and 45 g l^{-1} leaf extract recorded the highest ($P = 0.0169$) bursa weight (0.17, 0.18, 0.17, 0.17 and 0.17 %, respectively) while the lowest bursa weight (0.13 %) was obtained in hens in the control treatment. Statistically similar thymus mean weights recorded in hens administered 15, 30 and 45 g l^{-1} concentrations of root and leaf extract were significantly ($P = 0.0057$) higher than the values recorded for hens in the control groups. The highest ($P = 0.0323$) spleen weight was recorded in hens administered 15 and 45 g l^{-1} root extract (0.11 and 0.11 %, respectively) and 45 g l^{-1} leaf extract (0.12 %) while the lowest spleen weight was recorded in hens in the control treatment (0.09 %). Serum antibody titre against NDV increased with increasing concentration of both root and leaf extracts, and hens maintained on root or leaf extract at 30 and 45 g l^{-1} concentrations recorded the highest ($P < 0.0001$) antibody titre values (9.06 and 9.10 log_2, 9.08 and 9.18 log_2, respectively).

Liver sections from histopathological studies are presented in Figures 1–7. Liver sections of hens in all treatments appeared normal. The hepatocytes maintained normal shape, size and pattern.

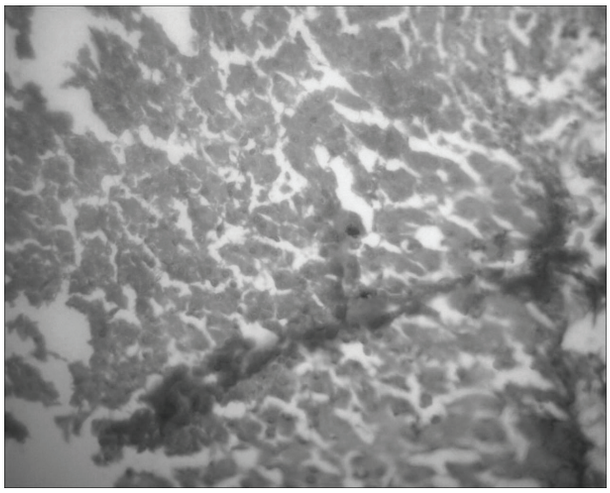

Fig. 1. Liver section of hen in the control treatment (0 g l^{-1} root/leaf extract)

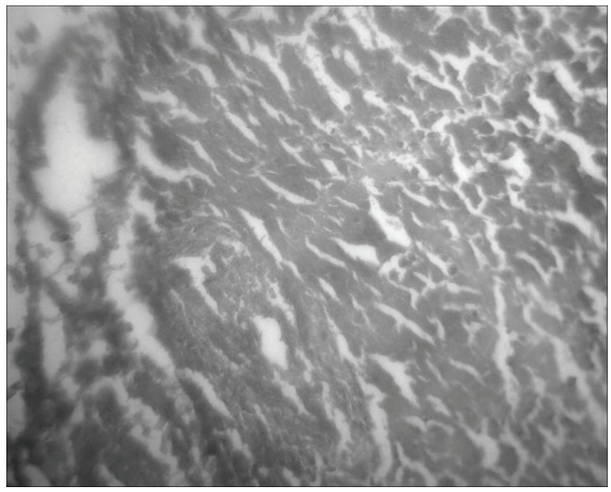

Fig. 2. Liver section of hen administered 15 g l^{-1} root extract

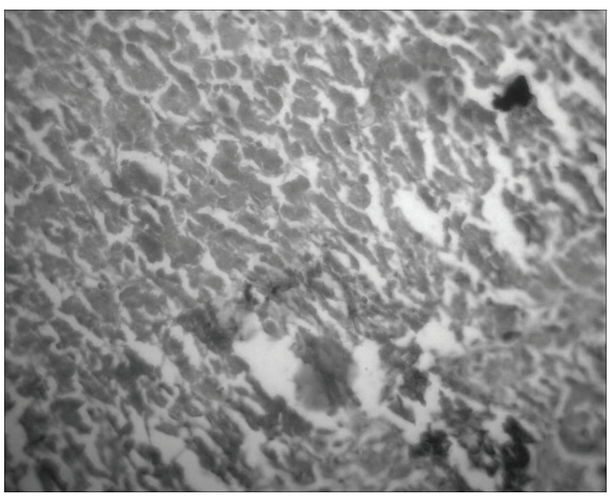

Fig. 3. Liver section of hen administered 15 g l^{-1} leaf extract

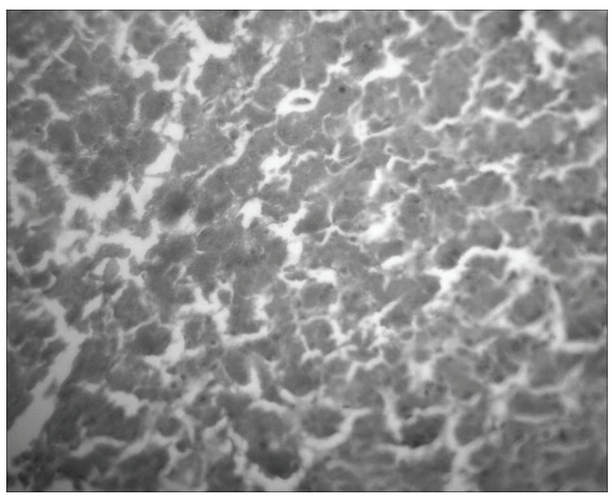

Fig. 4. Liver section of hen administered 30 g l^{-1} root extractt

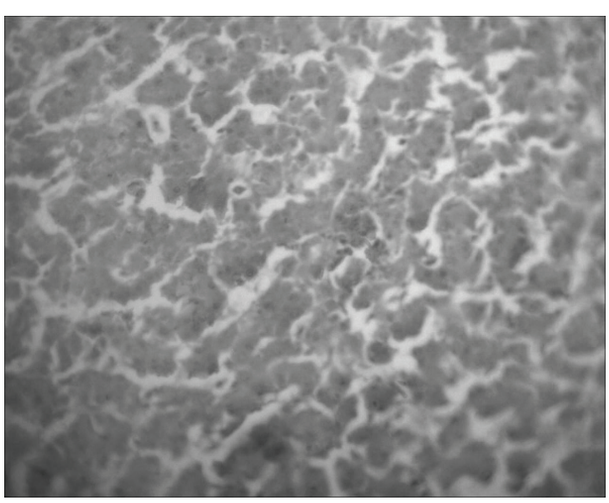

Fig. 5. Liver section of hen administered 30 g l^{-1} leaf extract

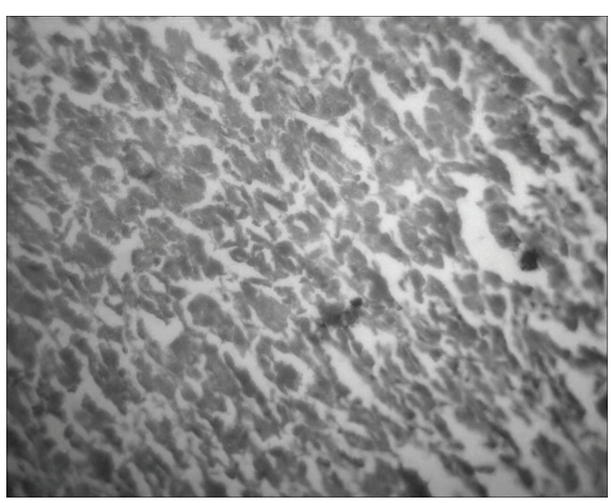

Fig. 6. Liver section of hen administered 45 g l^{-1} root extract

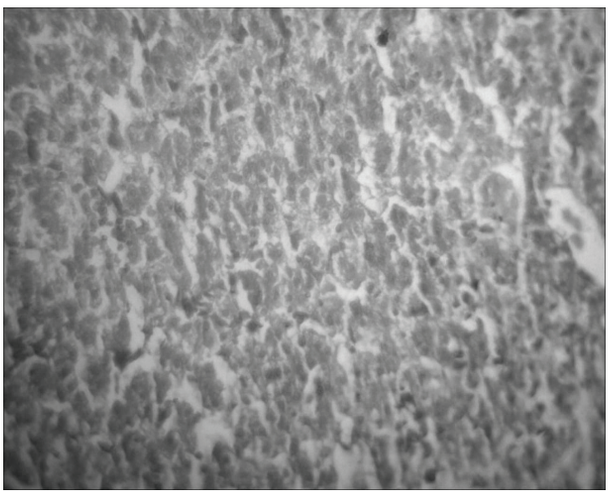

Fig. 7. Liver section of hen administered 45 g l^{-1} leaf extract

DISCUSSION

Reduction in faecal *Eimeria* oocyst count in hens administered *Petiveria alliacea* root or leaf extract at different concentrations indicated that the extracts were able to inhibit *Eimeria* parasite replication, thus can be effective against coccidiosis. Several bioactive compounds similar to those reported earlier (Ekunseitan et al., 2016) were isolated from *Petiveria alliacea* and most of these compounds possess antiparasitic activities. Alkaloid, a compound found in *Petiveria alliacea,* is capable of damaging DNA sequences in parasitic cells causing eventual death of the cells (Wink, 2012). Saponin present in *Petiveria alliacea* disrupts the cellular structure of parasitic cells leading to cellular death (Wang et al., 1998). Allicin, a sulphur compound found in garlic, which is similar to sulphur compounds isolated from *Petiveria alliacea* (de Andrade et al., 2012; Randle et al., 2018) was reported lethal to protozoan parasites (Al-Snaf, 2016). Strong antioxidants like phenols, flavonoids and tannins found in *Petiveria alliacea* (Balan et al., 2018) reduce the intensity of *Eimeria* infection by regulating lipid peroxidation process within the gut (Allen et al., 1998). The above activities might be responsible for the ability of *Petiveria alliacea* to inhibit *Eimeria* parasites. Although the information about the influence of *Petiveria alliacea* on *Eimeria* parasites is not available in literature, studies involving extracts of other medicinal plants have shown similar results (Gotep et al., 2016).

The observed reduction in intestinal bacteria counts in hens administered *Petiveria alliacea* root or leaf extract is an indication the plant has antibacterial qualities which can be attributed to the presence of several bioactive compounds. Dibenzyl-trisulfide found in *Petiveria alliacea* has shown strong antibacterial behaviour against numerous strains of bacteria (Kim et al., 2006; Randle et al., 2018). Other compounds such as tannins, alkaloids, flavonoids and phenols found in *Petiveria alliacea* are reportedly capable of destroying microbial cells by disintegrating their cell membrane and damaging their cellular structure (Barbieri et al., 2017). No literature was found on an *in-vivo* study of antimicrobial actions of *Petiveria alliacea* in poultry birds. However, the result of an *in-vitro* study (Ekunseitan et al., 2016) showed that the extract of *Petiveria alliacea* leaf had a higher antibacterial activity against some enteric pathogens affecting poultry species when compared with the fruit extract of *Lagenaria breviflora.* Similar antibacterial activities were reported in studies involving herbal plants possessing bioactive compounds similar to those present in *Petiveria alliacea*. Such a study by Jimoh et al. (2013) revealed that dietary supplementation of garlic powder significantly reduced caecal *Clostridium perfringens* load in broiler chickens. Sobayo et al. (2015) also observed a significant reduction in intestinal bacteria count of finisher broiler chickens fed 1 500 mg kg^{-1} dietary inclusion of neem leaf meal and 1 000 mg kg^{-1} dietary inclusion of garlic meal.

The observed increase in lymphoid organ weights of hens administered concentrations of *Petiveria alliacea* root extract or leaf extract suggested an increase in immunomodulatory activities within these organs. Bursa and thymus are involved in the production and orientation of immune cells (T-lymphocytes and B-lymphocytes) (Teo, Tan, 2007) while the spleen functions as a blood filter, removing pathogens circulating in the blood-stream (Lewis et al., 2019). A correlation between lymphoid organ weights and proliferation of immune cells indicating enhanced immunological activities was reported by Teo, Tan (2007). Therefore, immune enhancement activities of *Petiveria alliacea* (Randle et al., 2018) might have triggered a rise in immunological activities within these lymphoid organs and the subsequent increase in weight. Concentration-dependent rise in the antibody against NDV indicated that aqueous extracts of *Petiveria alliacea* root and leaf enhanced humoral immune defence mechanism of the hens by stimulating antibody production. Previous studies such as Alegre, Clavo (2007) identified dibenzyl-trisulfide found in *Petiveria alliacea* as an immune-stimulating agent. Williams et al. (1997, 2002) surmised that dibenzyl-trisulfide isolated from *Petiveria alliacea* increased thymic weight in mice while the histological study on the thymus revealed proliferation of cells. Quadros et al. (1999) reported the immuno-stimulatory effect of *Petiveria alliacea* in mice experimentally infected with *Listeria monocytogenes*. *In-vitro* and *in-vivo* studies revealed that water extract of *Petiveria alliacea* enhanced the production of lymphocytes, interferons and interleukins (Randle et al., 2018). Compounds such as phenols, flavonoids and terpenoids reportedly present in *Petiveria alliacea* preserved structural

integrity of immune cells and enhanced cellular and humoral immune defence mechanism (K a m b o h et al., 2015). There was no previous report on the immune-stimulatory effect of *Petiveria alliacea* on poultry birds. However, E l - k a t c h a et al. (2016) reported improvement in the immune response of broiler chicks fed diets supplemented allicin extracted from garlic which is similar to dibenzyl-trisulfide found in *Petiveria alliacea*. G a u t a m et al. (2017) also reported a higher antibody titre against the NDV antigen in broiler chickens supplied water supplemented with garlic paste compared with control birds.

The similarity in weight and normal appearance of liver tissues of hens in all treatments indicated that the administered concentrations of *Petiveria alliacea* root and leaf extracts were not hepatotoxic. However, O d e t o l a et al. (2019) observed hepatocellular necrosis in the liver of broiler chickens fed 1 500, 2 000 and 2 500 g inclusion of *Petiveria alliacea* root per 100 kg feed but the liver section of broiler chickens fed lower supplemental levels (500 and 1 000 g per 100 kg feed) appeared normal. The discrepancy in these results may be attributed to differences in the preparation method which affects the stability of bioactive compounds (P o o j a r y et al., 2017). It could also be probably attributable to differences in the dosage of administration.

CONCLUSION

The study concluded that the aqueous extract of *Petiveria alliacea* root and leaf at 30 and 45 g l^{-1} concentrations performed best as an antimicrobial and immune stimulating agent without impairing liver health.

REFERENCES

Alegre JC, Clavo M (2007): *Petiveria alliacea* L. Plant resources of tropical Africa. https://www.prota4u.org/database/protav8.asp?g=pe&p=Petiveria+alliacea+L. Accessed 29 March, 2020

Allen PC, Danforth HD, Augustine PC (1998): Dietary modulation of avian coccidiosis. International Journal of Parasitology, 28, 1131–1140. doi: 10.1016/s0020-7519(98)00029-0.

Al-Snaf AE (2016): Antiparasitic effects of medicinal plants (part 1) – A review. IOSR Journal of Pharmacy, 6, 51–56.

Attia G, El-Eraky W, Hassanein E, El-Gamal M, Farahat M, Hernandez-Santan A (2017a): Effect of dietary inclusion of a plant extract blend on broiler growth performance, nutrient digestibility, caecal microflora and intestinal histomorphology. International Journal of Poultry Science, 16, 344–353. doi: 10.3923/ijps.2017.344.353.

Attia G, Hassanein E, El- Eraky W, El-Gamal M, Farahat M, Hernandez-Santana A (2017b): Effect of dietary supplementation with a plant extract blend on the growth performance, lipid profile, immune response and carcass traits of broiler chickens. International Journal of Poultry Science, 16, 248–256. doi: 10.3923/ijps.2017.248.256.

Balan SG, Mohan VR, Muthukumarasamy S, Muthukrishna K (2018): *In vitro* antioxidant activity of *Petiveria alliacea* L. (Phytolaccaceae) and *Belloperone plumbaginifolia* (Acanthaceae). European Journal of Pharmaceutical and Medical Research, 5, 283–292.

Barbieri R, Coppo E, Marchese A, Daglia M, Sobarzo-Sanchez E, Nabavi SF, Nabavi SM (2017): Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. Microbiological Research, 196, 44–68. doi: 10.1016/j.micres.2016.12.003.

Bassiri E (2013): Enumeration of microorganisms. https://www.sas.upenn.edu/LabManuals/biol275/Table_of_Contents_files/14-Enumeration.pdf. Accessed March 5, 2020

Biswas AK, Kondaiah N, Anjaneyulu ASR, Mandal PK (2010): Food safety concerns of pesticides, veterinary drug residues and mycotoxins in meat and meat products. Asian Journal of Animal Sciences, 4, 46–55. doi: 10.3923/ajas.2010.46.55.

de Andrade TM, de Melo AS, Dias RG, Varela EL, de Oliveira FR (2012): Potential behavioural and pro-oxidant effects of *Petiveria alliacea* L. extract in adult rats. Journal of Ethnopharmacology, 143, 604–610.

Diarra MS, Malouin F (2014): Antibiotics in Canadian poultry productions and anticipated alternatives. Frontiers in Microbiology, 5, 282. doi: 10.3389/fmicb.2014.00282.

Duarte MR, Lopes JF (2005): Leaf and stem morphoanatomy of *Petiveria alliacea*. Fitoterapia, 76, 599–607. doi: 10.1016/j.fitote.2005.05.004.

Duncan DB (1955): Multiple range and multiple F tests. Biometrics, 11, 1–42. doi: 10.2307/3001478.

Ekunseitan DA, Yusuf AO, Olayinka OA, Ayoola A, Adedotun A (2016): Comparative study of two plants (*Lagenaria breviflora* and *Petiveria alliacea*) and their phytobiotic potential in poultry health. Nigerian Journal of Animal Production, 43, 289–298.

El-katcha MI, Soltan MA, Sharaf MM, Hasen A (2016): Growth performance, immune response, blood serum parameters, nutrient digestibility and carcass traits of broiler chicken as affected by dietary supplementation of garlic extract (Allicin). Alexandria Journal of Veterinary Sciences, 49, 50–64. doi: 10.5455/ajvs.219261.

Evans WC (2000): Trease and Evans' Pharmacognosy. WB Saunders Company Ltd.

Gautam G, Shrestha N, Bhandari S (2017): Effect of *Allium sativum* on immune status against Newcastle disease virus and productive performance of broiler chicken. International Journal of Poultry Science, 16, 515–521. doi: 10.3923/ijps.2017.515.521.

Gonzalez RM, Angeles HJ (2017): Antibiotic and synthetic growth promoters in animal diets: review of impact and analytical methods. Food Control, 72, 255–267. doi: 10.1016/j.foodcont.2016.03.001.

Google Earth (2019): Google Earth 7.3.2.5776, Google LLC, Mountain View CA.

Gotep JG, Tanko JT, Forcados GE, Muraina IA, Ozele N, Dogonyaro BB, Oladipo OO, Makoshi MS, Akanbi OB, Kinjir H, Samuel AL, Onyiche TE, Ochigbo GO, Aladelokun OB, Ozoan HA, Viyoff VZ, Dapuliga CC, Atiku AA, Okewole PA, Shamaki D, Ahmed MS, Nduaka CI (2016): Therapeutic and safety evaluation of combined aqueous extracts of *Azadirachta indica* and *Khaya senegalensis* in chickens experimentally infected with *Eimeria* oocysts. Journal of Parasitology Research, 2016, 1–9.

Harborne JB (1973): Photochemical methods: A guide to modern techniques of plant analysis. Chapman and Hall, London.

Jelveh K, Rasouli B, Seidavi A, Diarra SS (2018): Comparative effects of Chinese green tea (*Camellia sinensis*) extract and powder as feed supplements for broiler chickens. Journal of Applied Animal Research, 46, 1114–1117.

Jimoh AA, Ibitoye EB, Dabai YU, Garba S (2013): *In vivo* antimicrobial potentials of garlic against *Clostridium perfringens* and its promotant effects on performance of broiler chickens. Pakistan Journal of Biological Sciences, 16, 1978–1984. doi: 10.3923/pjbs.2013.1978.1984.

Kamboh AA, Arain MA, Mughal MJ, Zaman A, Arain ZM, Soomro AH (2015): Flavonoids: Health promoting phytochemicals for animal production: A review. Journal of Animal Health and Production, 3, 6–13. doi: 10.14737/journal.jahp/2015/3.1.6.13.

Kilany OE, Youssef F, Mabrouk M, Fares IM (2018): Clinicopathological studies on the effect of some antibacterial medicinal plants in broilers. Journal of Clinical Pathology Forecast, 1, 1003.

Kim S, Kubec R, Musah RA (2006): Antibacterial and antifungal activity of sulfur-containing compounds from *Petiveria alliacea* L. Journal of Ethnopharmacology, 104, 188–192. doi: 10.1016/j.jep.2005.08.072.

Lewis SM, Williams A, Eisenbarth SC (2019): Structure and function of the immune system in the spleen. Science Immunology, 4, 1–12. doi: 10.1126/sciimmunol.aau6085.

Muhammad SB, Sobayo RA, Oso AO, Sogunle OM, Ayoola AA, Adeyemo YO, Basiru YT (2019): Effects of dosage and plant parts of *Petiveria alliacea* used as phytobiotics on growth, nutrient digestibility and blood profile of pullet chicks. Archivos de Zootecnia, 68, 524–533.

NRC (1994): Nutrient requirements of poultry. 9th Ed. The National Academies Press, Washington D.C.

Nodu MB, Okpeku M, Akpoveta ZA, Iroegbu DO (2016): Evaluation of *Azadirachta indica* leave extract on hematology and biochemical profiles, organs weight and growth parameters of broiler chickens. Journal of New Sciences, 32, 1879–1884.

Odetola OM, Adejinmi OO, Owosibo OA, Banjo OT, Awodola-Peters OO (2019): Growth response, serum biochemistry and organ histopathology of broilers fed diets supplemented with graded levels of *Petiveria alliacea* root meal. International Journal of Poultry Science, 18, 45–50. doi: 10.3923/ijps.2019.45.50.

OIE (2008): Newcastle disease. Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees), chapter 2.3.14. https://www.oie.int/doc/ged/D7710.PDF. Accessed 5 March, 2020

Poojary M, Putnik P, Kovacevic D, Barba F, Lorenzo J, Dias D, Shpigelman A (2017): Stability and extraction of bioactive sulfur compounds from Allium genus processed by traditional and innovative technologies. Journal of Food Composition and Analysis, 61, 28–39. doi: 10.1016/j.jfca.2017.04.007.

Quadros MR, Souza-Brito ARM, Queiroz MLS (1999): *Petiveria alliacea* L. extract protects mice against *Listeria monocytogenes* infection – effects on bone marrow progenitor cells. Immunopharmacology and Immunotoxicology, 21, 109–124. doi: 10.3109/08923979909016397.

Randle MM, Riley CK, Williams LAD, Watson CTA (2018): A systematic review of the traditional and medicinal uses of *Petiveria alliacea* L. in the treatment of chronic diseases. Journal of Plant Science and Research, 5, 179–185.

Sahoo N, Mishra SK, Swain RK, Acharya A, Pattnaik S, Sethy K, Sahoo L (2019): Effect of turmeric and ginger supplementation on immunity, antioxidant, liver enzyme activity, gut bacterial load and histopathology of broilers. The Indian Journal of Animal Sciences, 89, 774–779.

Schmelzer G, Gurib-Fakim A (2008): Medicinal plants 1. Backhuys Publishers, Wageningen.

Slaoui M, Fiette L (2011): Histopathology procedures: from tissue sampling to histopathological evaluation. Methods in Molecular Biology, 691, 69–82. doi: 10.1007/978-1-60761-849-2_4.

Sobayo RA, Muhammad SB, Sogunle OM, Adegbenjo AA, Adejola AY, Oso AO, Adeyemi OA (2015): Response of finishing broiler chickens to supplemental neem (*Azadirachta indica*) and garlic (*Allium sativum*) on oocyst count, bacteria count and gut morphology. Bulletin of Animal Health and Production in Africa, 63, 443–453.

Sobayo RA, Okonkwo IJ, Sanwo KA, Oso OA, Eruvbetine D, Oguntona EB, Muhammed SB (2017): Effect of dietary supplementation of guinea hen weed (*Petiveria alliacea*) leaf and root meals on nutrient utilization and intestinal morphology of finishing broiler chicken. Nigerian Journal of Animal Science, 19, 144–156.

Sobayo RA, Okonkwo IJ, Muhammad SB, Sanwo KA, Oso OA, Sogunle OM (2018a): Haematological and serum indices of finishing broiler chickens fed graded levels of guinea hen weed (*Petiveria alliacea*) parts. Bulletin of Animal Health and Production in Africa, 66, 299–311.

Sobayo RA, Muhammad SB, Oso AO, Sogunle OM, Ayoola AA, Oke EO, Abotinde RO (2018b): Influence of guinea hen weed (*Petiveria alliacea*) on growth performance, nutrient digestibility and blood indices of growing pullets. Malaysian Journal of Animal Science, 2, 53–71.

Sofowora A (1993): Medicinal plants and traditional medicines in Africa. John Wiley and Sons.

Teo AY, Tan HM (2007): Evaluation of the performance and intestinal gut microflora of broilers fed on corn-soy diets supplemented with *Bacillus subtilis* PB6 (CloSTAT). Journal of Applied Poultry Research, 16, 296–303. doi: 10.1093/japr/16.3.296.

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). Animal Feed Science and Technology, 74, 143–153. doi: 10.1016/S0377-8401(98)00137-0.

Williams LAD, The TL, Gardner M, Fletcher CK, Naravane A, Gibbs N (1997): Immunomodulatory activities of *Petiveria alliacea*. Phytotherapy Research, 11, 143–144. doi: 10.1002/(SICI)1099-1573(199705)11:3<251::AID-PTR75>3.0.CO;2-B.

Williams LAD, Rosner H, Conrad J, Moller W, Beifuss U, Chiba K (2002): Selected secondary metabolites from phytolaccaceae and their biological/pharmaceutical significance. Recent Research Development in Phytochemistry, 6, 13–68.

Williams LAD, Rosner H, Levy HG, Barton EN (2007): A critical review of the therapeutic potential of dibenzyl trisulphide isolated from *Petiveria alliacea* L. (guinea hen weed, anamu). West Indian Medical Journal, 56, 17–21. doi: 10.1590/S0043-31442007000100004.

Wink M (2012): Medicinal plants: A source of anti-parasitic secondary metabolites. Molecules, 17, 12771–12791. doi: 10.3390/molecules171112771.

Zajac AZ, Conboy GA (2012): Veterinary clinical parasitology. Wiley-Blackwell.

Corresponding Author:

Dr. Adetola M. O y e l e k e , Federal University of Agriculture Abeokuta, College of Animal Science and Livestock Production, Department of Animal Production and Health. P.M.B 2240, Alabata Road, Abeokuta, Ogun State, Nigeria,
e-mail: oyeadetola@outlook.com