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RESEARCH ARTICLE

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Antimicrobial, immunomodulatory and hepatomodulatory effects of aqueous extracts of *Petiveria alliacea* root and leaf on growing pullets

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Abstract

Aim of study: To evaluate antimicrobial, immunomodulatory and hepatomodulatory effects of aqueous extracts of *Petiveria alliacea* L. (Guinea hen weed) root and leaf on growing pullets.

Area of study: Abeokuta, Nigeria.

Material and methods: Two hundred and eighty eight eight-weeks-old Isa-brown pullets were divided into eight treatments which consisted in birds administered root extracts or leaf extracts at four different concentrations (0, 15, 30 and 45 g/L). Each treatment was replicated three times with 12 pullets per replicate.

Main results: Oocyst counts was lower ($p<0.05$) in pullets administered root extract (78.42 opg) compared with leaf extract (103.58 opg). Oocyst counts was lower ($p<0.05$) in pullets administered 30 and 45 g/L root or leaf extract compared with other treatments. Bacteria counts reduced ($p<0.05$) in pullets administered root extract compared with leaf extract. Pullets administered 45 g/L root extract recorded lowest ($p<0.05$) bacteria count. Bursa and thymus weights increased in pullets administered root or leaf extract compared with the control. Pullets administered 30 and 45 g/L root or leaf extract had highest ($p<0.05$) antibody titre against Newcastle disease vaccine 8.80, 8.86, 8.74 and 8.80 (log2) respectively. There was fatty infiltration in liver of control birds, while hepatocytes appeared normal in liver of pullets administered *P. alliacea* extracts.

Research highlights: P. alliacea root and leaf extracts at 45 g/L performed best as antimicrobial, immune-stimulating and hepato-protective agent in pullets. This study highlights P. allicaea as a valuable antimicrobial and immunostimulating agent in poultry production.

Additional key words: oocyst counts; bacteria count; liver histopathology; herbal plants; poultry production; Guinea hen weed

Abbreviations used: HI (haemagglutination inhibition); NDV (Newcastle disease vaccine);

Authors' contributions: Performed the experiments and wrote the paper: AMO. Supervised the work: OAA, LTE and RAS. All authors conceived and designed the experiments, analyzed the data and participated in critical revision of the manuscript.

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Introduction

Commercial layer enterprise is a common subsection within Nigeria poultry industry. The productive performance of commercial layers to a great extent depends on health management during their growing phase (Olawumi, 2011). Pullets raised in proper health condition tends to reach and maintain optimal productive performance during their laying phase while pullets raised with health challenges may be inconsistent in production or become unproductive at the laying phase (Olawumi, 2011). Many health challenges encountered in poultry production arise from bacteria and coccidial infections (Adene, 1996).

Bacterial infection is of great economic importance in poultry production. Many poultry diseases result from colonization of the gut by harmful bacteria which are often characterized by high bacteria load (Albazaz & Buyukunal Bal, 2014). Coccidiosis is a crucial and common disease in poultry production (Ola-Fadunsin & Ademola, 2014). It is characterized by invasion of the gut by *Eimeria* parasites leading to tissues damage, haemorrhage, weight loss and death (Ola-Fadunsin & Ademola, 2014; Eke *et al.*, 2016). Bacterial infections and coccidiosis significantly increase cost of production and causes huge economic loss in poultry industry (Ganguly & Praveen, 2016; Lawal *et al.*, 2016). Overtime, these diseases have been prevented by

the use of various conventional antibiotic and anticoccidial drugs (Aarestrup & Jenser, 2007; Thangarasu *et al.*, 2016).

Emergence of drug resistant bacteria and harmful drug residue in animal products has led to restriction in use of conventional drugs (Diarra & Malouin, 2014; Dhama *et al.*, 2015; Gonzalez-Ronquillo & Angeles-Hernandez, 2017). Moreover, there is increasing public awareness on harmful drug residue in animal products and necessity for food safety, leading to increase in demand for organic-based products (Biswas *et al.*, 2010). These developments necessitate search for viable substitutes to conventional drugs for growth promotion, prophylactic, therapeutic and immune enhancement purposes, readily available at low cost without detrimental effects.

An alternative gaining considerable recognition is the use of medicinal plants which are residue-free, readily available and potent against diseases (Diaz-Sanchez *et al.*, 2015). Several herbal materials have been studied for their prospective benefit as growth-promoting, prophylactic, therapeutic and immune-enhancing agents. Many of these studies have reported positive and promising results (Molan & Faraj, 2015; Nghonjuyi, 2015; Attia *et al.*, 2017a,b; Navidshad *et al.*, 2018). However, the use of medicinal plants in poultry production has not been fully established and investigation on herbal materials is still in progress in order to fully explore the benefits of available medicinal plants to the poultry industry.

Petiveria alliacea L. is a potent herb whose medicinal benefits to the poultry industry have not been explored. *P. alliacea* belongs to the family Phytolaccaceae, the herb is native to North, Central and Southern America and the Caribbean with wide spread introduced population in Nigeria (Schmelzer & Gurib-Fakim, 2008; USDA-ARS, 2020). The plant has been used successfully in folk medicine to treat various ailments and conditions (Brown, 1995; Randle *et al.*, 2018). Scientific investigations showed that the plant possess antibiotic, anti-oxidant, anti-carcinogenic, anti-cancerous, anti-inflammatory and immuno-modulatory properties (Kim *et al.*, 2006; Williams *et al.*, 2007; Santander *et al.*, 2012; Pacheco *et al.*, 2013; Ekunseitan *et al.*, 2016; Oluwa *et al.*, 2017). Some researchers (Sobayo *et al.*, 2017, 2018a,b; Muhammad *et al.*, 2019; Odetola *et al.*, 2019) have investigated the growth and performance enhancement abilities of *P. alliacea* on poultry species. However, there is no substantial information on therapeutic benefits of the plant to poultry species. Hence, this study investigated the antimicrobial, immunomodulatory and hepatomodulatory effects of aqueous extracts of *P. alliacea* root and leaf on growing pullets.

Material and methods

This experiment was carried out according to animal welfare guidelines of Animal Welfare Committee, College of Animal Science and Livestock Production, Federal University of Agriculture Abeokuta, Nigeria. The study was carried out at the research unit of Livelihoods Support and Development Centre (SLIDEN AFRICA), Abeokuta, Ogun State, Nigeria.

Extraction of *Petiveria alliacea* root and leaf

Aqueous extraction was carried out according to the method outlined by Nodu *et al.* (2016). Fresh roots and leaves of *P. alliacea* were sourced from Kotopo area of Abeokuta, Ogun State, Nigeria. The roots and leaves were rinsed in clean water to remove adhering dirt. Using a sensitive weighing scale, 15, 30 and 45 g samples of fresh roots and leaves were taken and each sample was blended separately in 1-L of clean water. Each blended mixture was separated by filtration using 0.1 mm sieve screen. The root and leaf particles were discarded while filtrate from each mixture was presented to experimental birds as drinking water according treatment.

Experimental management

A total of 288 eight-weeks-old Isa brown pullets were used for this study which lasted for 10 weeks. The pullets were arranged in a 2×4 factorial experimental layout in a completely randomised design. There were eight experimental treatments replicated three times with 12 birds per replicate. The treatments consisted in birds administered aqueous extract of *P. alliacea* root at 0 g/L (control), 15 g/L, 30 g/L and 45 g/L concentrations and leaf at 0 g/L (control), 15 g/L, 30 g/L and 45 g/L concentrations. The extracts were presented to pullets via drinking water on two consecutive days per week throughout the experiment. Extracts presented met and exceeded daily water requirement of the birds on all occasions of administration. Experimental birds were raised in a deep litter system and fed grower mash diet formulated according to National Research Council recommendation (Table 1). Pullets in all treatments were free of antibiotic and anticoccidial drugs throughout the experiment.

Data collection

Fourteen days prior to the end of the experiment, four birds were randomly selected from each replicate of all treatments. These birds were housed in a separate cage on treatment and replicate basis and administered Newcastle Disease Vaccine (NDV) lasota strain via drinking water for future serum antibody test. Routine administration of *P. alliacea* extracts was maintained

Table 1. Composition of the experimental diet

Ingredients	(%)
Maize	40.00
Soya bean meal	5.00
Fish meal (72% CP)	1.50
Groundnut cake	5.00
Palm kernel cake	18.50
Wheat offal	26.80
Oyster shell	1.00
Bone meal	1.50
Lysine	0.10
Methionine	0.10
Common salt	0.25
Premix[1]	0.25
Total	100.00
Calculated analysis	
Dry matter (%)	88.90
Crude protein (%)	15.50
Crude fibre (%)	7.50
Ether extracts (%)	3.45
Ash (%)	6.22
Metabolisable energy (MJ/kg)	11,513.70

CP: crude protein. [1] Premix (composition per kg diet): Vitamins B1, B2, B6, 0.02 g; Vitamin K3, 3 g; Vitamin E, 30 g; biotin, 0.05 g; folic acid, 1.5 g; choline chloride, 250 g; nicotinic acid, 30 g; Ca-pantothenate, 15 g; Co, 0.4 g; Cu, 8 g; Fe, 32 g; I, 0.8 g; Zn, 40 g; Mn, 64 g; Se, 0.16 g; butylated hydroxytoluene, 50 g.

Faecal *Eimeria* oocyst count

Anticoccidial effect was estimated by *Eimeria* oocyst counts in faeces of experimental birds. At the end of the experiment (18 weeks of age), dropping trays covered with aluminium foil were placed on the floor of each rearing unit. Fresh faecal droppings were picked from each replicate of all treatments into separate labelled sterile bottles using sterile forceps. The bottles were placed in ice and transported to the laboratory for egg count analysis using McMaster egg counting technique as described by Zajac & Conboy (2012).

Intestinal total bacteria count

Antibacterial effect was estimated by evaluating intestinal total bacteria count in experimental birds. At the end of the experiment, live bodyweights of birds selected earlier were determined after which they were slaughtered. Small intestines of the slaughtered birds were carefully isolated from the carcass and placed in separate labelled sterile bottles. The bottles were placed in ice and transported to the laboratory for bacterial count. Total bacteria count was carried out on mixed digesta from the small intestine using viable count method as described by Bassiri (2013). The colony-forming units (cfu) were expressed as logarithm to base 10.

Lymphoid organ weight and serum antibody against Newcastle disease vaccine

Immunomodulatory effect was estimated by evaluating weight of lymphoid organs and serum antibody titre against NDV.

Lymphoid organs (bursa, thymus and spleen) were carefully isolated from the carcass of slaughtered birds, weighed using a sensitive weighing scale and expressed as percentage of live bodyweight.

Serum antibody titre against NDV was determined by haemagglutination inhibition (HI) test. Blood (2 mL) was collected aseptically from each slaughtered bird into labelled plain bottles. The blood samples were allowed to clot and the sera separated into different labelled sterile bottles. The samples were transported to the laboratory for HI test using the method described by OIE (2008). The HI titre values were expressed as logarithm to base two.

Liver weight and histopathological examination

Hepatomodulatory effect was evaluated by liver weight and histopathological examination. Livers of slaughtered birds were weighed using a sensitive weighing scale and expressed as percentage of live bodyweight. The livers were fixed in 10 % formalin solution and processed for histopathological examination according to Slaoui & Fiette (2011). Hepatic changes were viewed with a BA410E Elite Research Compound Microscope and photomicrograph taken with aid of an Amscope MU900 digital camera.

Statistical analysis

The experiment was arranged in a 2×4 factorial experimental layout in a completely randomized design. Data collected were subjected to 2-way Analysis of Variance, significant ($p<0.05$) differences between treatment means were determined using Duncan Multiple Range Test as contained in Statistical Analysis Software (SAS, 2010) package.

The model of the study was:

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

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

Results

Table 2 presents the main effects of the aqueous extracts of *P. alliacea* parts (root and leaf) and concentration of extraction on faecal *Eimeria* oocyst counts and intestinal total bacteria counts in growing pullets. Faecal *Eimeria* oocyst count was significantly (p<0.05) lower in birds administered root extract (78.42 opg) compared with bird administered leaf extract (103.58 opg). Intestinal total bacteria count was reduced (p<0.05) by administration of root extract compared with leaf extract. Furthermore, faecal *Eimeria* oocyst count reduced significantly from 197.50 opg in the control treatment to 21.33 opg at 45 g/L concentration of extraction. Intestinal total bacteria count reduced significantly as concentration of extraction increased.

Table 3 presents the interactive effect of aqueous extracts of *P. alliacea* parts (root and leaf) and concentration of extraction on faecal *Eimeria* oocyst counts and intestinal total bacteria counts in growing pullets. *Eimeria* oocyst counts were significantly (p<0.05) lower in pullets administered 15, 30 and 45 g/L root and leaf extract than values obtained in the control treatments. Pullets administered 45 g/L root extract and 45 g/L leaf extract recorded lower (p<0.05) intestinal total bacteria counts (0.83 and 1.20 log10 (cfu/g) respectively) than the control treatments (1.83 and 2.00 log10 (cfu/g) respectively).

Table 4 presents the main effects of aqueous extracts of *P. alliacea* parts (root and leaf) and concentration of extraction on liver weight, lymphoid organ weights and serum antibody against NDV. Bursa weight was significantly (p<0.05) higher in pullets maintained on 15, 30 and 45 g/L concentrations than value obtained in the control treatment. The highest (p<0.5) thymus weight (0.48 %) was recorded in pullets maintained on 45 g/L concentration while the lowest (0.37 %) was recorded in the control treatment. Spleen weight was higher (p<0.05) in pullets maintained on 45 g/L concentration (0.15 %) compared with the control treatment (0.12 %). Serum antibody titre against NDV increased (p<0.05) from 7.05 (log2) in control birds to 8.83 (log2) in birds maintained on 45 g/L concentration.

Table 5 presents the interactive effect of aqueous extracts of *P. alliacea* parts (root and leaf) and concentration of extraction on liver weight, lymphoid organ weights and serum antibody against NDV. Bursa weights were statistically similar in pullets administered 15, 30 and 45 g/L of *P. alliacea* root and leaf extracts but the values were higher (p<0.05) compared with the control treatments. Thymus weight was significantly (p<0.05) higher in pullets administered 15, 30 and 45 g/L root extract and 30 and 45 g/L leaf extract compared with the control treatments. Serum antibody titres against NDV were statistically similar in pullets administered 30 and 45 g/L concentration of

Table 2. Main effects of aqueous extracts of *Petiveria alliacea* parts (root and leaf) and concentrations of extraction on faecal *Eimeria* oocyst count and intestinal total bacteria count in growing pullets

	Plant parts		SEM	*p*-value	Concentrations of extraction (g/L)				SEM	*p*-value
	Root	Leaf			0	15	30	45		
Faecal *Eimeria* count (opg)	78.42^{b}	103.58 ^{a}	22.08	0.0104	197.50 ^{a}	107.83 ^{b}	37.33 ^{c}	21.33c	10.70	<0.0001
Intestinal bacteria count (log^{10} (cfu/g))	1.30^{b}	1.58 ^{a}	0.13	0.0482	1.92^{a}	1.47 ^{b}	1.35 ^{bc}	1.02 c	0.13	0.0017

SEM: standard error of mean. opg: oocysts per gram. ^{a,b,c}: means in the same row not sharing common superscript by factor are significantly (p<0.05) different.

Table 3. Interactive effect of aqueous extracts of *Petiveria alliacea* parts (root and leaf) and concentrations of extraction (0, 15, 30, 45 g/L) on faecal Eimeria oocyst count and intestinal total bacteria count in growing pullets

	Root				Leaf				SEM	*p*-value
	0	15	30	45	0	15	30	45		
Faecal *Eimeria* count (opg)	200.00^{a}	72.33 ^{c}	21.33 ^{d}	20.00 ^{d}	195.00 ^{a}	143.33 ^{b}	53.33 ^{cd}	22.67 ^{d}	11.11	<0.0001
Intestinal bacteria count (log^{10} (cfu/g))	1.83^{ab}	1.30 ^{bcd}	1.23 ^{bcd}	0.83 ^{d}	2.00 ^{a}	1.64 ^{abc}	1.47 ^{abc}	1.20 ^{cd}	0.17	0.0087

SEM: standard error of mean. opg: oocyst per gram. ^{a,b,c,d}: means in the same row not sharing common superscript are significantly (p<0.05) different

Table 4. Main effects of aqueous extracts of *Petiveria alliacea* parts (root and leaf) and concentrations of extraction on liver weight, lymphoid organ weights and serum antibody against Newcastle Disease Vaccine (NDV)

	Plant parts		SEM	*p*-value	Concentrations of extraction (g/L)				SEM	*p*-value
	Root	Leaf			0	15	30	45		
Liver (%)	1.55	1.53	0.05	0.7780	1.49	1.55	1.58	1.54	0.07	0.8708
Bursa (%)	0.20	0.20	0.01	0.5410	0.17^{b}	0.20^{a}	0.21^{a}	0.22^{a}	0.01	0.0001
Thymus (%)	0.43	0.42	0.01	0.6755	0.37^{c}	0.41^{b}	0.44^{b}	0.48^{a}	0.01	<0.0001
Spleen (%)	0.14	0.13	0.01	0.4836	0.12^{b}	0.14^{ab}	0.13^{ab}	0.15^{a}	0.01	0.0464
Antibody titre against NDV (log^2)	8.25	8.23	0.22	0.7384	7.05^{c}	8.32^{b}	8.77^{a}	8.83^{a}	0.06	<0.0001

SEM: standard error of mean. ^{a,b,c}: means in the same row not sharing common superscript by factor are significantly (p<0.05) different. Values presented for liver, bursa, thymus and spleen are expressed as percentage of the live body weight of slaughtered hens

Table 5. Interactive effect of aqueous extracts of *Petiveria alliacea* parts (root and leaf) and concentrations of extraction extraction (0, 15, 30, 45 g/L) on liver weight, lymphoid organ weights and serum antibody against Newcastle Disease Vaccine (NDV)

	Root				Leaf				SEM	*p*-value
	0	15	30	45	0	15	30	45		
Liver (%)	1.51	1.58	1.54	1.57	1.48	1.52	1.61	1.51	0.10	0.9845
Bursa (%)	0.17^{b}	0.20^{a}	0.20^{a}	0.22^{a}	0.17^{b}	0.20^{a}	0.21^{a}	0.21^{a}	0.01	0.0012
Thymus (%)	0.38^{de}	0.43^{abcd}	0.42^{bcd}	0.48^{a}	0.36^{e}	0.40^{cde}	0.45^{abc}	0.47^{ab}	0.02	0.0008
Spleen (%)	0.12	0.14	0.13	0.15	0.12	0.13	0.13	0.15	0.01	0.2611
Antibody titre against NDV (log^2)	7.07^{c}	8.30^{b}	8.80^{a}	8.86^{a}	7.03^{c}	8.34^{b}	8.74^{a}	8.80^{a}	0.10	<.0001

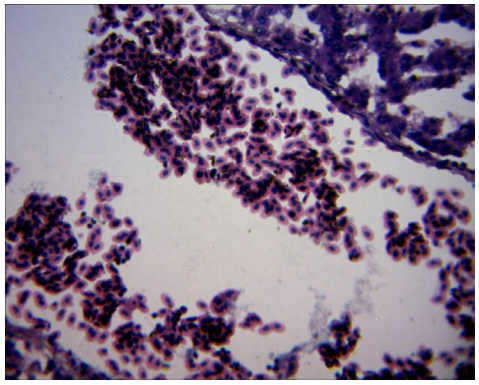
SEM: standard error of mean. ^{a,b,c,d,e}; means in the same row not sharing common superscript are significantly (p<0.05) different. Values presented for liver, bursa, thymus and spleen were expressed as percentage of the live body weight of slaughtered hens.

P. alliacea root and leaf extracts (8.80, 8.86, 8.74 and 8.80 log2 respectively) and the values were higher (p<0.05) compared with other treatments.

Histopathological sections of liver of pullets in all experimental treatment are shown in Figures 1. Histopathological examination of liver of pullets in the control treatment revealed hepatic fatty infiltration. However, liver sections of pullets administered 15, 30 and 45 g/L concentrations of *P. alliacea* root and leaf extracts appeared normal, the hepatocytes maintained normal size and arrangement.

Discussion

Reduction in *Eimeria* oocyst counts in faeces of pullets administered extracts of *P. alliacea* root and leaf at various concentrations indicated that the plant is capable of inhibiting *Eimeria* parasite replication in growing pullets. Several bioactive compounds effective against parasitic cells have been isolated from *P. alliacea* (Ekunseitan *et al.*, 2016). Saponin which was found in the plant was reported to cause death of parasitic cells by disrupting their cellular structure (Wang *et al.*, 1998). Alkaloid present in the plant was reported to prevent parasitic cell replication by damaging their DNA sequence leading to eventual cellular death (Wink, 2012). Sulphur compound found in garlic (allicin), which is similar to sulphur compound isolated from *P. alliacea* (Randle *et al.*, 2018), has been reported to be toxic to a wide range of protozoan parasites (Al-Snaf, 2016). Moreover, Ekunseitan *et al.* (2016) reported the presence of tannins, flavonoids and phenolics in *P. alliacea*; these compounds were identified as strong antioxidants (Balan *et al.*, 2018) which help in moderating lipid peroxidation processes within the gut, thereby reducing the intensity of *Eimeria* infection (Allen *et al.*, 1998). These biological processes could have inhibited the *Eimeria* parasite's replication. Further reduction in oocyst count at higher concentrations of extraction indicated that Eimeria parasite replication is responsive to the concentration of phytochemicals present in the extracts administered to pullets. Lower faecal oocyst count recorded in pullets administered root extract compared with leaf extract suggested that the bioactive compounds are present at higher concentration in the root compared with the leaf. Although information about the influence of *P. alliacea* on *Eimeria* parasites in chickens is not available in literature, similar results were reported in studies involving other medicinal herbs. Combined aqueous extracts of *Azadirachta indica* A. Juss. and *Khaya senegalensis* (Desr.) A. Juss. reduced faecal oocyst count in broiler birds infected with Eimeria species in a dosage dependent manner (Gotep *et al.*, 2016). Ola-Fadunsin & Ademola (2013) observed a concentration-dependent reduction in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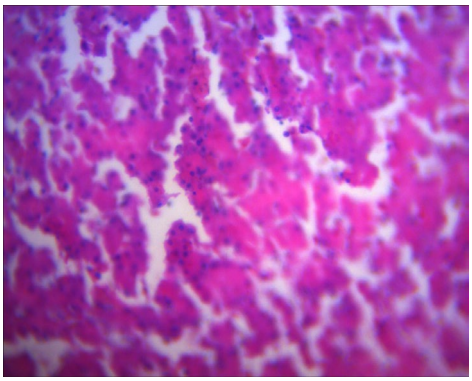
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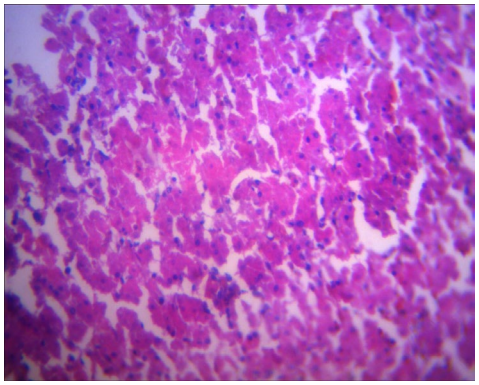
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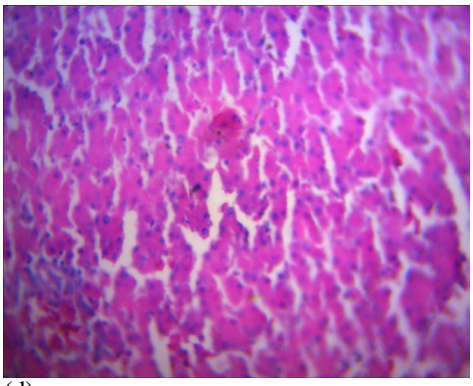
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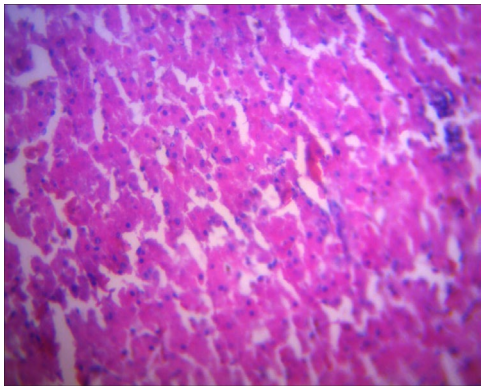
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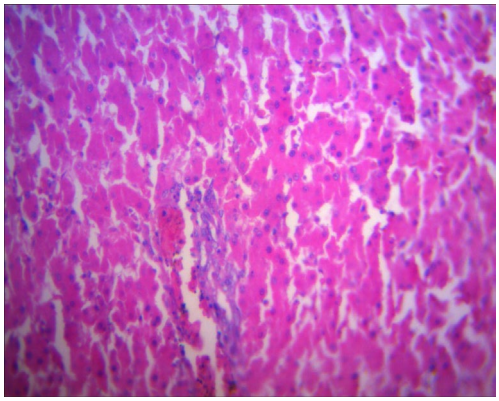
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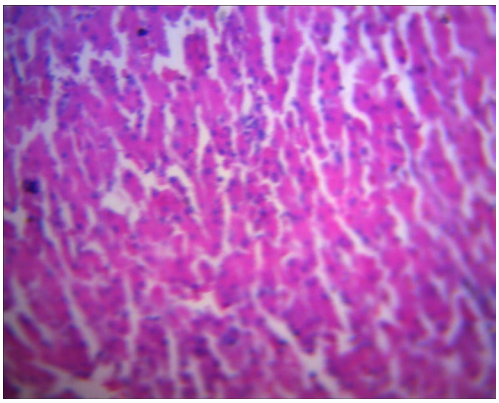
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(g)

Figure 1. Histopathological sections of liver of experimental birds. (a) Control treatment showing fatty infiltration of hepatocytes (arrow). Pullets administered: (b) 15 g/L aqueous extract of *Petiveria alliacea* root, (c) 15 g/L aqueous extract of *P. alliacea* leaf, (d) 30 g/L aqueous extract of *P. alliacea* root, (e) 30 g/L aqueous extract of *P. alliacea* leaf, (f) 45 g/L aqueous extract of *P. alliacea* root, and (g) 45 g/L aqueous extract of *P. alliacea* leaf, all showing normal size and arrangement of hepatocytes.

oocyst shedding in broiler chickens infected with *Eimeria* parasite and treated with acetone extract of *Moringa oleifera* Lam. leaf.

This study confirmed the antibacterial activity of *P. alliacea* by reduction in intestinal total bacteria count observed in pullets administered aqueous root and leaf extracts. This antibacterial activity can be attributed to the presence of various bioactive compounds identified as potent antibiotic agents in the plant. Ekunseitan *et al.* (2016) and Adesipo *et al.* (2017) isolated phytochemicals such as terpenoids, flavonoids, tannins, alkaloids, phytate, phenols, saponin, oxalate, carotenoids and sulphur-containing compounds from *P. alliacea*. The sulphur-containing compounds (benzyl-containing thiosulfinates) isolated from the plant have been reported to exhibit strong antimicrobial ability and as the major constituent contributing to the antimicrobial property of the plant (Kim *et al.*, 2006; Randle *et al.*, 2018). Moreover, flavonoids, tannins, alkaloids and phenols were reported to cause death of microbial cells by damaging their cellular structure (Cowan, 1999; Barbieri *et al.*, 2017). The concentration-dependent reduction in intestinal bacteria count signified further suppression of bacteria growth and replication when higher concentrations of the bioactive compounds were administered. Lower intestinal bacteria count in pullets administered root extract compared with leaf extract implied that antimicrobial compounds were present in the root at higher concentration compared with the leaf. This corresponds with the finding of Kim *et al.* (2006), who stated that precursors (cysteine sulfoxides) to the major antimicrobial compound (dibenzyl trisulfide) in the *P. alliacea* are present at higher concentration in the root compared with the leaf. No previous study has presented result on antimicrobial effect of *P. alliacea* on poultry species. However, in an *in-vitro* study, Ekunseitan *et al.* (2016) concluded that extract of *P. alliacea* leaf inhibited some bacteria pathogen important to poultry species. Related results were presented in studies involving herbal materials having similar bioactive compounds as *P. alliacea.* Dietary supplementation of garlic powder reduced caeca *Clostridium perfringens* population in broiler chickens (Jimoh *et al.*, 2013). Feeding garlic meal at 1000 mg/kg inclusion and neem leaf meal at 1500 mg/kg inclusion reduced intestinal bacteria count in broiler chickens (Sobayo *et al.*, 2015).

Bursa and thymus are primary lymphoid organs in chicken responsible for the production and orientation of immune cells, T-lymphocytes and B-lymphocytes (Teo & Tan, 2007). Spleen is a secondary lymphoid organ useful in filtering out pathogens in the blood (Lewis *et al.*, 2019). Changes in weight of lymphoid organs could affect the ability of animals to maintain productivity during sanitary challenges (Eyng *et al.*, 2015). Increase in lymphoid organ weight correlates with enhanced proliferation of immune cells which represents better immunity (Teo & Tan, 2007). The observed increase in lymphoid organ weights of pullets administered extracts of *P. alliacea* root and leaf suggested an increase in activities within these organs triggered by the immunomodulatory ability of the plant. It also indicated better proliferation of immune cells thus an enhancement in immune system. The concentration-dependent increase in antibody titre against NDV seen in pullets administered extracts of *P. alliacea* root and leaf also signified the ability of the plant to strengthen the immune system by increasing antibody production against foreign bodies. The immune-enhancement ability of *P. alliacea* can be attributed to bioactive compounds present in the plant. Phenols, part of the compounds found in *P. alliacea,* were found to maintain structural integrity of immune cells (Awaad *et al.*, 2010). Flavonoids and terpenoids enhance humoural and cellular immunity and regulate the endocrine and circulatory markers of health (Abdulkarimi, 2011; Kamboh *et al.*, 2015). Dibenzyl-trisulfide found in *P. alliacea* was also identified as an immunomodulatory compound (Alegre & Clavo, 2007). Earlier studies found that dibenzyl-trisulfide increased thymic weight in mice and histological analysis on the thymus revealed there was proliferation of cells (Williams *et al.*, 1997, 2002). In *in-vitro* and *in-vivo* studies water extract of *P. alliacea* was reported to enhance lymphocyte, interferon and interleukin production (Randle *et al.*, 2018). Quadros *et al.* (1999) also reported an immune-stimulatory effect of *P. alliacea* on mice infected with *Listeria monocytogenes*. No literature was found on immunomodulatory effect of *P. alliacea* on poultry species however, studies on garlic which possess similar bioactive compound as *P. alliacea* have produced similar results (El-katcha *et al.*, 2016; Gautam *et al.*, 2017).

In the present study, hepatoprotective activity was displayed by *P. alliacea* by maintaining the structural integrity of hepatocytes. Antioxidant compounds such as flavonoids, saponin, terpenoids and tannins (Zhang *et al.*, 2015) found in *P. alliacea* were reported to stabilise reactive oxygen species thereby inhibiting oxidative damage and degenerative changes in hepatocytes (Ojo *et al.*, 2015). The result also suggested hypolipidemic effect of the plant considering hepatic fatty infiltration observed in liver of control birds which was absent in *P. alliacea* treated birds. Flavonoids and saponins found in *P. alliacea* were reported to prevent excessive fat synthesis by moderating activities of enzymes involved in lipogenesis within the liver (Borradaile *et al.*, 2003; Patel *et al.*, 2012) which might be responsible for the observed hypolipidemic property. However, Odetola *et al.* (2019) reported that *P. alliacea* root meal at high supplemental levels (1500, 2000 and 2500 g per 100 kg feed) induced hepatocellular necrosis in broiler chickens but these signs were absent at lower supplemental levels (500 and 1000 g per 100 kg feed). This means that methods of preparation which can affect stability of active compounds in plants (Poojary

et al., 2017) as well dosage of administration can influence hepatoprotective activities of *P. alliacea*.

In conclusion, 45 g/L concentration of *P. alliacea* root and leaf extracts performed best as anticoccidial, antibacterial and immune-stimulating agent without impairing liver health. The study encouraged further studies to determine effect of *P. alliacea* on individual *Eimeria* and bacteria species affecting poultry birds.

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