Assessment of Guinea hen weed (*Petiveria alliacea* L.) Leaf Extracts on Blood indices and Oxidative status in Broiler Chickens

[1]Bamigbola, I. P., [1,2]Ekunseitan, D. A., [1]Adeshina E. T., [1]Akinade, T. M., [1]Akodu, M. T., [1]Jubril, I. E, [1]Jimoh, A. S., [1]Adegun, W. O., and [2]Adebayo, O. J.

[1]Department of Animal Production and Health, Federal University of Agriculture, Abeokuta, Ogun state, Nigeria

[2]Department of Animal Sciences, North Carolina Agricultural and Technical State University, Greensboro, NC, USA.

***Corresponding author**: ekunseitanda@funaab.edu.ng

Abstract

Oxidative stress poses a serious challenge in broiler production, leading to the development of natural health promoting alternatives such as Petiveria alliacea due to its antioxidant benefit to conventional treatments. A total of 192, 1-day old Cobb500 broiler chicks were assigned to four treatments: Control (0.25 mL of 20% Enrofloxacin), 200 mL, 400 mL, and 600 mL of air-dried Petiveria alliacea extract per 1000 mL of drinking water in a completely randomized design. The chicks were raised in a deep litter system. Each treatment had 48 chicks per group. Data on haematological and serum metabolites indices, and blood oxidative stability were, subjected using one way analysis of variance (ANOVA). Results revealed that red blood cells values were lower ($p>0.05$) in birds given 600 mL of oral Petiveria alliacea leaf extracts. Albumin values were however elevated ($P<0.05$) in chicks administered 200 and 600 mL of PA extracts while higher creatinine levels were observed in chicks on 400 and 600 mL PA concentrations. A reduction in malondialdehyde (MDA) concentrations was observed at 400 mL of Petiveria alliacea extracts when compared with other treatments, while glutathione peroxidase was higher at 600 mL PA extracts ($P<0.05$). It can be concluded that the administration of Petiveria alliacea leaf extracts could be a veritable tool in mitigating against the adverse effect of oxidative stress without causing deleterious effects on the blood indices of broiler chickens at 600 mL per litre of water of PA extracts.

Keywords: *Petiveria alliacea*, blood profile, oxidative stability, broiler chickens.

Évaluation des extraits de feuilles d'herbe à poule (*Petiveria alliacea* L.) sur les indices sanguins et le statut oxydatif chez les poulets de chair

Résumé

Le stress oxydatif représente un défi sérieux dans la production de poulets de chair, conduisant au développement d'alternatives naturelles favorables à la santé telles que Petiveria alliacea en raison de ses bienfaits antioxydants par rapport aux traitements conventionnels. Un total de 192 poussins de chair Cobb500 d'un jour ont été répartis en quatre traitements : Témoin (0,25 mL d'Enrofloxacine à 20 %), 200 mL, 400 mL et 600 mL d'extrait de Petiveria alliacea séché à l'air par 1000 mL d'eau de boisson, selon un dispositif complètement randomisé. Les poussins ont été élevés en système de litière profonde. Chaque traitement comptait 48 poussins par groupe. Les données sur les indices hématologiques et métaboliques sériques, ainsi que la stabilité oxydative du sang, ont été soumises à une analyse de variance à un facteur (ANOVA). Les résultats ont révélé que les valeurs des globules rouges étaient plus basses ($p>0,05$) chez les oiseaux recevant 600 mL d'extrait oral de feuilles de Petiveria alliacea. Les valeurs d'albumine étaient cependant élevées ($P<0,05$) chez les poussins recevant 200 et 600 mL d'extrait de PA, tandis que des niveaux de créatinine plus élevés ont été observés chez les poussins recevant 400 et 600 mL de concentrations de PA. Une réduction des concentrations de malondialdéhyde (MDA) a été observée à 400 mL d'extrait de Petiveria alliacea par rapport aux autres traitements, tandis que la glutathion peroxydase était plus élevée à 600 mL d'extrait de PA ($P<0,05$). On peut conclure que l'administration d'extraits de feuilles de Petiveria alliacea pourrait être un outil efficace pour atténuer les effets néfastes du stress oxydatif sans causer d'effets délétères sur les indices sanguins des poulets de chair à la dose de 600 mL par litre d'eau d'extrait de PA.

Mots-clés : *Petiveria alliacea*, profil sanguin, stabilité oxydative, poulets de chair.

Introduction

The use of ethnomedicinal herbs in livestock production in the treatment of infectious and inflammatory diseases has been of immense benefit in the health management of poultry birds (Ekunseitan *et al.,* 2016; Yusuf *et al.,* 2023). This indispensable source of medicine used for the prevention and treatment of conventional diseases by small scale farmers, also, has found it relevance in commercial system of poultry production due to the presence of active bioactive metabolites (Dhama *et al.,* 2015; Ekunseitan *et al.,*2016; Yusuf *et al.,* 2023). Despite the aforementioned positive benefits, a direct effect of plant extracts on blood and antioxidant potential has been reported. The strategic use of plant extracts and the inherent metabolites in counteracting the adverse impact of inflammation in livestock as part of the immune response to infections, injuries and oxidative stress capability of activating the white blood cells leading to inflammation is being encouraged over the conventional system of production since the latter has significantly impacted the prevalence of toxicity and gene resistance microbes in the gastrointestinal tract (Yasunari *et al.,* 2002; Ekunseitan *et al.,* 2023). The influence of antibiotics can directly interact with reactive oxygen metabolites and affect inflammation levels which can, in turn, influence oxidative stress (Mourenza *et al.,* 2020). Which this has caused a widespread ban by most countries on the usage and administration of antibiotics by different regulatory bodies such as European Union and the National Agency for Food and Drug Administration and Control. This resultant effect is geared at bridging the gap between poultry health and food safety.

The use of active biological metabolites and physiological effect of phytobiotics are gradually recognized to regulate and altered different functions of inflammatory response in poultry birds either directly or indirectly (Valko *et al.,* 2007). These various inflammatory responses are made up of complex immune system consisting of well-organized set of cells interacting in a group with a definite function, especially for the purpose to protect the body system from diseases (Soomro, 2019). However, one of the challenges in the broiler sector is obtaining suitable specific secondary plant metabolites to mitigate against inflammations due to complex biological responses of vascular tissues to pathogenic and non-pathogenic antigens, along the production line (Soomro, 2019). This problem ultimately reduces growth and feed efficiency, susceptibility to disease, nutrient metabolism, immune system activation and economic losses (Soomro, 2019). During the pathological and physiological conditions, there is an increased production of free non-oxygen radicals and reactive oxygen metabolites because the red blood cells is very prone to biological damage due to the high cell concentration of oxygen and haemoglobin, a very powerful promoter of oxidative process (Bryszewska *et al.,* 1995; Georgieva *et al.,* 2006). Red blood cells and other inflammatory cells are equipped with effective systems of antioxidants that offered protection against oxidative damages to tissues and organs in the body (Siems *et al.,* 2000; Arbos *et al.,* 2008). However, certain amount of oxidative stress is important to the body for growth and cell signaling, but overproduction of reactive oxygen metabolites have been reported to inhibit and damage the normal functions of lipids, proteins and impair gut function (Khansari *et al.,*2009; Pandey and Rizvi, 2011). Recently, ascorbic acid, alpha-tocopherol, selenium, and plant species such as curcuma, anise, ginger, coriander that are greatly abundant in carotenoids, anthocyanins and flavonoids has been proposed and use as a novel exogenous antioxidant additive in combating oxidative stress with limited side effect (Kikusato, 2021; Alagawany, *et al.,* 2022), therefore broadening the spectrum of seeking alternative plant compounds that is high in antioxidant and anti-inflammatory properties targeted at fighting reactive oxygen metabolites is required.

In this regard, the potential of *Petiveria alliacea* as an effective ethnomedicinal plants cannot be overemphasized in Nigeria. The plant extracts have been widely used extensively, for the treatment of different diseases such as skin infection, toothache and diabetes in human ethnomedicine. However, the intrinsic potential of *Petiveria alliacea* leaf extract showing its anti-inflammatory effects has been reported due to its high levels of antioxidants. Therefore, this is a

good alternatives antibiotic for poultry health needed to maintains productivity and ensure better product integrity (Ekunseitan *et al.,* 2016; Zhang *et al.,* 2024). The phytochemical screening and gas chromatography-mass spectrometry of the different components of *Petiveria alliacea* leaf extracts revealed the concentration of phytoconstituents possesses numerous pharmacological activities such as antioxidant, anti-inflammatory, analgesics, antimicrobial, anti-diabetic, anti-hypertensive and several other activities (Ekunseitan *et al.,* 2016; Adeyemi *et al.,* 2017). Most of the abundant broad spectrum biological metabolites contained in the extracts include steroids, sulfurs, flavonoids, alkaloids, triterpenoids, saponins, glycosides, carotenoids, tannins (Ekunseitan *et al.,* 2016; Arogbodo *et al.,* 2021). Dipropyl disulphide, dibenzyl trisulphide, dibenzyl sulphide, dibenzyl disulphide, benzylhydroxymethyl sulfide and di (benzyltrithio) methane have all be discovered and reported in the leaves of *Petiveria alliacea* (Benevides *et al.,* 2001). In addition, the therapeutic evaluation of *Petiveria alliacea* has been attributed to its potential for several anti–inflammatory and antioxidants properties exhibited to have a suppressive effect on the development of reactive oxygen metabolites to hinder the lipoperoxidation chain reaction (Ekunseitan *et al.,* 2016; Olomieja *et al.,* 2021; Adesanya *et al.,* 2023). The degree of lipid peroxidation is usually used as an indication of free radical-induced wounds (Kuun and Borchert, 2002), and the malondialdehyde level in blood and tissue is generally used as a biomarker of lipid peroxidation (Yousef *et al*., 2009). The medicinal effects of *Petiveria alliacea* for the prevention of liver injury and restoration of antioxidant status by root meal in broiler birds supplemented with graded levels of *Petiveria alliacea* root meal have been reported (Odetola *et al.,* 2019). The antigenotoxic potential based on plasmid protection induced by the extract of *Petiveria alliacea* against oxidative damage was reported by Soares *et al.* (2014). The presence of tannins; flavonoids and its derivatives such as leridal-chalcone, flavanone types leridal, leridol, leridol-5-methyl ether, flavonol types myricitrin, benzyl-2-hydroxyethysulfide, isoarborinol, isoarborinol astetat, and isoarborinol cinnamate, its content in the tested leaves is suggestive of its antioxidant and anti-inflammatory benefits as these metabolites has been reported as potent antioxidant by increasing the activity of endogenous antioxidants enzymes (Gunawan *et al.,* 2020). The leaves of *Petiveria alliacea* is reported to be high in lipids contents such as lignoceryl lignocerate, linoleic acid, nonadecanoic acid, palmitic acid and stearic acid (Raintree Nutrition, 2013). However, there is no information on the leaf extracts on blood indices and oxidative status in broiler chickens. Therefore, the study was conducted the assess effective administration of *Petiveria alliacea* leaf extracts on blood indices and oxidative status in broiler chickens.

Materials and Methods

The experiment was conducted at the Poultry Department, Directorate of University Farms, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Harvested *Petiveria alliacea* leaf were separated from the petiole, washed thoroughly with clean water to remove sand and dirt, and then air-dried at constant moisture content. The aqueous extract of *Petiveria alliacea* was obtained by infusing 100 g of the air-dried *Petiveria alliacea* leaf per litre of hot boiling water and subsequently allowed to cool for one hour. The extracts were filtered with a sieve while the filtrate was collected and stored in an air-tight container for further use. 500g leaves per 5 L of hot boiling water better expressed as 500g/L of boiling water. One hundred and ninety-two, day-old broiler chicks were assigned to four treatments: Control (0.25 mL of 20% Enrofloxacin), 200 mL, 400 mL, and 600 mL of *Petiveria alliacae* extract per 1000 mL of water in a completely randomized design. Each treatment had 48 chicks per group. The prepared aqueous extract of *Petiveria alliacea* was administered to the bird for three consecutive days per week while ordinary water was given on other days of the week throughout the experiment. The birds were fed commercial-based diets consisting of starter feed (Energy: 30000kcal/kg; Crude protein: 22%) and finisher feed (Energy: 3100kcal/kg; Crude protein: 18%).

Table 1: Gross Nutritional Composition of Diet Fed to Broiler Chickens

Analysed nutrient levels	Starter	Finisher
Metabolizable Energy (kcal/kg)	3000.00	3100.00
Crude Protein (%)	22.00	18.00
Ether Extract (%)	6.00	6.00
Crude Fibre (%)	5.00	5.00
Calcium (%)	1.00	1.00
Available Phosphorus (%)	0.45	0.40
Lysine (%)	1.00	0.85
Methionine (%)	0.50	0.34
Sodium (%)	0.30	0.30

Data Collection

Estimation of Blood and Serum Metabolites

About 6 mL of blood was collected from the wing vein of each bird in all treatments on the 42nd day of the experiment into two separate labeled bottles for blood and serum examination. The 3 mL of the drawn blood was decanted into a pretreated Ethylene Diamine Tetra Acetic acid (EDTA) bottle to prevent coagulation. Packed cell volume and red Blood count were determined as described by the micro-haematocrit and haemocytometer method (Thrall and Weiser, 2002). White blood count was estimated based on the Wintrobe haematocrit tube according to the procedure of Schalm (Jain, 1986). Leucocyte differential counts such as lymphocyte, heterophil, eosinophil, basophil, and monocyte were analyzed using Wright stain to detect the differential counts as outlined by Ritchie *et al.* (1994). The remaining blood was emptied into plain bottles for some serum metabolite determination. Total protein was determined by the direct Biuret method (Lubran, 1978; Varley *et al.* 1980), and Albumin by the bromocresol green method (Doumas and Biggs, 1971). The serum concentration of globulin was calculated as the difference between total protein and albumin concentrations. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to standard procedures by Reitman and Frankel, 1957.

Determination of Blood Oxidative Stability

On the 42nd day of the experiment, 3 mL of blood was collected from one selected broiler bird in each replicate with the use of a hypodermic needle and syringe via the jugular vein into a labeled Ethylene Diamine Tetra-acetic acid (EDTA) bottle. Catalase (CAT) was determined according to Levesque (2008). Superoxide dismutase (SOD) was determined using the procedure of Oberley *et al.* (1998), malondialdehyde (MDA) was determined according to Siu and Draper (1978), and Glutathione peroxidase (GSH-Px) was determined using the procedure of Reddy *et al.* (2004).

Statistical Analysis

Data obtained were subjected to One-way analysis of variance (ANOVA) in a completely randomized design by SAS (2009). Significant differences among means were separated using the Duncan's multiple range test at 5% level of significance**.**

Results

All blood metabolites measured were not significantly ($p>0.05$) affected. However, red blood cells had the lowest ($p>0.05$) non-significant effect value in birds administered 600 mL of oral *Petiveria alliacea* leaf extract.

Table 2: Effects of Aqueous Extracts of *Petiveria alliacea* Leaves on the Blood Metabolites of Broiler Chickens

Parameters	**0**	**200**	**400**	**600**	**SEM**	**P-value**
Packed Cell Volume (%)	31.75	30.00	31.25	26.50	0.95	0.203
Haemoglobin (g/dL)	10.43	9.93	10.45	8.85	0.32	0.266
Red Blood Cell (10^6/uL)	3.43	3.40	3.31	2.79	0.11	0.107
White Blood Cell (/10^3/uL)	0.15	0.14	0.17	0.14	0.36	0.430
Lymphocytes (%)	62.00	59.50	61.75	58.75	1.04	0.654
Heterophils (%)	31.50	33.50	30.75	34.50	1.10	0.631
Monocytes (%)	2.75	3.00	3.25	3.06	0.21	0.847
Eosinophils (%)	3.25	3.75	4.00	3.25	0.42	0.916
Basophil (%)	0.50	0.25	0.25	0.25	0.12	0.673

SEM- Standard error mean

The effect of the aqueous extracts of *Petiveria alliacea* leaves on the serum indices of broiler chickens is presented in Table 4. The oral administration of *Petiveria alliacea* leaf extract significantly ($p<0.05$) influenced the level of albumin and creatinine while other parameters were not affected ($p>0.05$). Serum albumin in chicks administered 200 and 600 mL of the extracts was similar and higher ($p<0.05$) than levels observed in other treatments while those on 400 and 600 mL *Petiveria alliacea* leaf extracts had similar serum creatinine concentrations

.

Table 3: Effects of Aqueous Extracts of *Petiveria alliacea* Leaves on the Serum Indices of Broiler Chickens

Parameters	Control	200	400	600	SEM	P-value
Aspartate aminotransferase (I.U/L)	77.80	88.64	77.74	83.83	4.60	0.833
Alanine aminotransferase (I.U/L)	2.32	2.90	1.90	4.12	0.50	0.365
Alkaline phosphatase (I.U/L)	266.20	258.50	276.30	265.64	3.20	0.281
Total protein (g/dL)	2.79	2.51	2. 58	2.79	0.20	0.916
Albumin (g/dL)	1.24^{c}	1.70^{a}	1.63^{b}	1.70^{a}	0.10	0.033
Creatinine (mg/dL)	0.40^{b}	0.47^{b}	0.57^{a}	0.59^{a}	0.30	0.036
Globulin (g/dL)	1.55	0.81	0.95	1.09	0.15	0.345

$^{abc:}$ Means in the same row differ significantly ($p<0.05$)

SEM- Standard error mean

The effect of oral administration of aqueous extracts of *Petiveria alliacea* on blood oxidative stability of broiler chickens is presented in Table 5. Aqueous extract of *Petiveria alliacea* had no significant effect ($p>0.05$) on blood oxidative stability measured except malondialdehyde and Glutathione peroxidase. The highest ($p<0.05$) malondialdehyde was observed in the 200 mL group while the lowest value was recorded in the control and 400 mL group respectively. Glutathione peroxidase was highest ($p<0.05$) in the 600 ml group compared to other groups.

Table 4: Effect of Oral Administration of Aqueous Extracts of *Petiveria alliacea* Leaves on Blood Oxidative Stability of Broiler Chickens

Parameter	0	200	400	600	SEM
Catalase (U/L)	0.59	1.07	0.58	1.79	0.2956
Superoxide dismutase (U/L)	0.008	0.007	0.009	0.008	0.0004
Malondialdehyde (U/L x 10^{-9})	0.87^{c}	1.52^{a}	0.88^{c}	1.28^{b}	0.1137
Glutathione peroxidase (U/L)	6.56^{b}	5.98^{c}	6.24^{b}	8.57^{a}	0.6378

^{abc} Means with different superscripts along the same row are significantly different ($p<0.05$

Discussion

The use of ethnomedicinal herbs has been recognized globally not only for the promotion of health but bridging the gap in fighting the severity of pathogenic oxidative stress, while maintaining stable ecological oxidative balance (Georgieva *et al*., 2006). In the present study, the blood indices (PCV, Hb RBC, and WBC) obtained in the study were within the normal range observed by previous researchers in chickens (Bounous and Stedman, 2000). This signifies that the level of *Petiveria alliacea* administered to broiler chickens could be tolerated without affecting the haemopoetic activity in the blood system. Sobayo *et al.* (2018) and Oyeleke *et al.* (2021) however observed significantly higher red blood cells in growing pullets served on a diet supplemented with *Petiveria alliacea* root meal. This may be attributed to the administration route and age of birds. Despite higher albumin levels obtained at 200 and 600 mL *Petiveria alliacea* extract yet, the result obtained was within the tolerable level in the tropic of 1.17 – 2.74 g/dL (Meluzzi *et al.,* 1992). Albumin level has been linked with the normal functioning of the liver (Adeyemi *et al.,* 2012). The albumin concentration is a useful marker for the secretory and excretory function of the liver (Suliaman *et al.,* 2014), enhanced osmotic balance, transporting nutrients and immune function. The increased level of albumin could be associated with the presence of Sulphur compounds in *PA* extracts with the capacity of sulfur to serve as a precursor in protein biosynthesis (Oguntoye *et al.,* 2018). This study did not align with the report of Odetola *et al.* (2019) who observed a non-significant effect in albumin of broiler chickens fed diets supplemented with graded levels of *Petiveria alliacea* root meal at 5, 10, 15, 20, and 25g/kg of feed throughout 8 weeks. The creatinine levels of this study were above the normal (0.10 – 0.40 mg/dL) range established by Merck (2012). The highest creatinine values were obtained in 400 and 600 mL *Petiveria alliacea* concentrations. The values obtained were within the tolerable level as documented by researchers in the tropic. Okorie *et al.* (2011) and Okereke *et al.* (2019) observed 0.90 – 2.00 mg/dL and 0.35 -1.67 mg/dL for normal broiler birds. This result suggests that the concentration of secondary metabolites at those levels was not detrimental to the renal functionality of the broiler birds (Oni *et al.,* 2018). Inadequate utilization and excretion of waste products such as muscle contraction and protein metabolism in the blood is an indication of renal damage which can result in weight loss and health problems in broiler birds (Kolawole *et al.,* 2017). Owosibo *et al.* (2013) observed higher levels of creatinine for broiler chick diets containing probiotics. Duwai *et al.* (2013) had increased creatinine value in broiler birds in comparison to the result obtained for this study.

Glutathione peroxidase (GSH-Px) is a selenoprotein (Xianyong *et al*., 2017) that protects susceptible substrates by eliminating free radical oxygen ions and hydrogen peroxide initiators and propagators by transferring hydrogen atoms to stabilize the free radicals to impede lipid peroxidation (Aguilar *et al*., 2007). The increase in Glutathione peroxidase in broiler chickens administered 600 mL concentration of *Petiveria alliacea* leaf extract could be a direct influence of the sulfur-containing amino acid, dibenzylthiosulfinate known as petivericin and sulfenic acid, which can efficiently form a line of defense against reactive oxygen metabolites and activate the antioxidant capacity by lowering lipid peroxidation due to lower stearic hindrance, reported to be among the fastest antioxidant (Amorati *et al*., 2012; 2019). The possible mechanism of action of the secondary metabolites such as sulfenic acids in *Petiveria alliacea* leaf extract are important intermediates in cellular redox regulation formed by the oxidation of thiol functions in cysteine residues and can be converted into thiosulfinates and disulfides (non-phenolic antioxidants) innate ability to quench reactive oxygen metabolites and augment the levels of important endogenous antioxidants (Roos and Messens, 2011; Amorati *et al.,* 2012; 2019). Flavonoids and several phenolic compounds have been observed as inhibitors of lipid peroxidation thereby limiting the formation of free radicals that lead to oxidative stress (Shtukmaster *et al.,* 2010). Malondialdehyde (MDA) is an important indicator of oxidative stress and the formation of lipid peroxidation due to attacks by free radicals on the cell membrane of unsaturated fatty acids (Mirghaed *et al*., 2019) and important biomarkers of oxidative stress because of lipid peroxidation. (Ayala *et al.,* 2014). This process of interaction

between lipid peroxidation and free radicals leads to the uptake of electrons from the lipids membrane resulting in biological damage to the cell. Oxidative stress is a product of aldehydes such as malonialdehyde and 4- 4-hydroxynonenal (Ayala *et al.,* 2014). The significant reduction observed in MDA of broiler chickens administered 400 mL *Petiveria alliacea* extract might be attributed to the presence of flavonoid and tannin compounds by lowering the elevation of MDA in which free radicals are reduced (Bone and Mills, 2013). Flavonoids act directly as antioxidants, protecting the cells from free radicals and also increasing the activity of antioxidant enzymes (Abdelmoaty *et al.,* 2010). Tannins not only enhance the activity of endogenous antioxidants but also increase antioxidant levels. Tannins also reduce MDA concentration in the liver, heart, and kidney of broiler birds (Velayutham *et al.,* 2012). The significant increase in GSH-Px at 600 mL oral administration of *Petiveria alliacea* extract might be the antioxidant potential in the blood serum counteracting the concentration of MDA, indicative of reduced oxidative stress and lipid peroxidation (Mirghaed *et al*., 2018). The resultant relationship between GSH-Px and MDA production is proportionate to the levels of the antioxidant enzyme by clearly indicating the important role of GSH-Px as a potent antioxidant defense enzyme against the concentration of MDA in the blood of broiler birds (Ekunseitan *et al*., 2021).

Conclusion

Oxidative stress impairs health status disposing birds to disease infections, therefore, the use of *Petiveria alliacea* leaf extracts as effective and potential natural antioxidant at 600 mL per litre of drinking water without affecting health and productivity of the birds.

References

Adesanya, E. O., Oyesiku, O. O., Adesanya, O. O., Ogunlakin, A. D., Odugbemi, A. I., &Egieyeh, S. A. 2022. Phytochemical components and GC and MS analysis of *Petiveria alliacea L.* fraction and volatile oils. *Researchgate.* 1:13. https://doi.org/10.1515/psr-2022-0311

Abdelmoaty, M. A., Ibrahim, M. A., Ahmed, N. S., & Abdelaziz, M. A. 2010. Confirmatory studies on the antioxidant and antidiabetic effect of quercetin in rats. *Indian Journal of Clinical Biochemistry.* 25(2): 188-192. DOI:10.1007/s12291-010-0034-x

Ad Yemi, O. S., Akanji, M. A., &Ekanem, T. O. 2012. Ethaolic extract of *Psidium guajava* influences protein and bilirubin levels in *Trypanosoma brucei* infected rats. *Journal of Biologica Science.* 12: 111-116.

Ad Yemi, M. A., Ekunseitan D. A., Abiola, S. S., Dipeolu, M. A., Egbeyale, L. T., & Sogunle, O. M. 2017. Phytochemical analysis and GC-MS determination of *Lagenaria breviflora* R. fruit. *International Journal of Pharmacognosy and Phytochemical Research.* 9(7): 1051-1056. http://doi:10.25258/phyto.v9i07.11178.

Ag uilar, A., Alvarez-Vijande, R., Capdevila, S., Alcoberro, J., & Alcaraz, A. 2007. Antioxidant patterns (superoxide dismutase, glutathione reductase and glutathione peroxidase) in kidneys from non-heart beating-donors: experimental study. *Transplant Proc.* 39(1): 249-252.

Alagawany, M., Ashour, E. A., El-Fakhrany, H. H. H., Ismail, T. A., & Nasr, M. 2022. Early nutrition programming with *Astragalus membranaceus* polysaccharide: its effect on growth, carcasses, immunity, antioxidants, lipid profile and liver and kidney functions in broiler chickens. *Animal Biotechnology*. 33(2):362-368. Doi:10.1080/10495398.2021.2025067.

Am orati, R., Lynett, P. T., Valgimigli, L., & Pratt, D. A. 2012. The reaction of sulfenic acid with peroxyl radicals: insights into the radical trapping antioxidant activity of plant-derived thiosulfinates. *Chemistry*. 63: 37-40.

Amorati, R., Baschieri, A., & Valgimigli, L. 2019. The role of sulfur and heavier chalcogens in the chemistry of antioxidants. *Phosphorus, Sulfur, and Silicon and the Related Elements.* ISSN:

1042-6507. https://doi.org/10.1080/10426507.2019.1602620.

Ar bos, K. A., Claro, L. M., Borges, L., Santos, C. A., & Weffort-Santos, A. M. 2008. Human erythrocytes as a system for evaluating the antioxidant capacity of vegetable extracts. *Nutrition Research.* 28:457-63.

Arogbodo, J. O., Igbe, F. O., Osho, I. B., & Adebayo, I. A. 2021. Processing yield percentage and phytochemical appraisement of the leaf, stem-bark, inflore-infructescence, and root of *Petiveria alliacea* (Linneaus). *International Journal of Science Research Biology Science.* 8:65-70.

Ayala, A., Munoz, M. F., &Arguelles, S. 2014. Lipid peroxidation products in human health and disease: Production, metabolism and signling mechanisms of malondialdehyde and 4-hyroxy-2-nonenal. *Journal of oOxidative Medicine and Cellular Longevity.* Article ID 360438. https://doi.org/101155/2014/360438.

Benevides, P., Young, M., Giesbrecht, A., Roque, N., &Bolzani, V. 2001. Antifungal polysulphides from *Petiveria alliacea* L. *African Journal of Traditional, Complementary Alternative Medicine.* 1394:436-44.

Bone, K., and Mills, S. 2013. How to use the monographs. In M. Dixon, and Blumenthal, M. (Eds), Principles and practices of phytotherapy: Modern herbal medicine pp. 381-961. Edinburgh: Churchill Livingstone.

Bounous, D. I., & Stedman, N. L. 2000. Normal avian haematology: chicken and turkey, In: Freldman, B.F., Zinkl, J.G., Jain, N.C. Schalm's Veterinary Heamatology, 5th edition. Lippincott, Williams and Wilkisin, Philadelphia, pp: 1147-1154.

Bryszewska, M., Zavodnik, I.B., Niekurzale, A., & Szosland, K. 1995. Oxidative processes in red blood cells from normal diabetic individuals. *Biochemical Molecular Biology International.* 37:345-54.

Dhama, K., Latheef, S. K., Mani, S., Abdul Samad, H., Karthik, K., Tiwari, R., Khan, R. U., Alagawany, M., Farag, M. R., Alam, G. M., Laudadio, V., & Tufarelli, V. 2015. Multiple beneficial applications and modes of action of herbs in poultry health and production – A review. *International Journal of Pharmacology.* 11(3):152-176.

Doumas, B. T., &Biggs, H. G. 1971. Determination of serum albumin, in GR Cooper edition, Standard methods of clinical chemistry, New York, USA. Pp. 7-175.

Duwai, J., Saleh, B., &Adegbolah, T. A. 2013. Heamatological and serum biochemical characteristics of cockerels fed graded level of boiled sorrel seed meal. *Online Journal of Animal and Feed Research.* 2: 111-115.

Ekunseitan, D. A., Yusuf, A. O., Olayinka, O. A., Ayoola, A. A., & Adegbenjo, A. A. 2016. Comparative study of two plants (*Lagenaria breviflora* and *Petiveria alliacea*) and their phytobiotic potential in poultry health. *Nigeria Journal of Animal Production.* 289-298.

Ekunseitan, D. A., Yusuf. A. O., Ekunseitan, O. F., Alao, S. O., &Allinson, A. Z. 2021. Dietary supplementation of vitamin E and selenium on performance and oxidative stability of meat of broiler chickens in a hot climate. *Agricultura Tropica et Subtropica*. 54(1): 24-31. https://doi.org/10.2478/ats-2021-0003.

Ekunseitan, D. A., Akinola, M. O., Adegun, W. O., Akinwunmi, E. O., & Omoseyin, T. M. 2023. Quality attributes and antioxidant activities of meat of broiler chickens administered aqueous *Lagenaria breviflora. Agricultura Tropica et Subtropica*. 56:74-80. Doi: 10.2478/ats-2023-0009.

Georgieva, N. V., Koinarski, V., & Gadjeva, V. 2006. Antioxidant status during the course of *Eimeria tenella* infection in broiler chickens. *The Veterinary Journal.* 172:488-492.

Gu nawan, V. A., Soetjipto, H., & Mustika, A. 2020. Hypoglicemic and antioxidant activity of *Petiveria alliacea* in diabetic

rat models. *Biomolecular and Health Science Journal.* 3(1):19-23.

Jain, N. C. 1986. Schalm's veterinary haematology, 4th Edition. Lea and Febiger.

Khansari, N., Shakiba, Y., & Mahmoudi, M. 2009. Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. *Recent Patentson Inflammation and Allergy Drug Discovery.* 3;73-80.

Kikusato, M. 2021. Phytobiotics to improve health and production of broiler chickens: Functions beyond the antioxidant activity. *Animal Bioscience.* 34(3). DOI:10.5713/ab.20.0842.

Kolawale, T. A., & Dapper, D. V. 2017. Gastro-protective effects of the methanolic extract of the rind of *Citrullus lanatus* on indomethacin induced gastric ulceration in male Wistar rats. *Acta Pharmaceticeutical Science.* 55:4. https://doi.org/10.23893/1307-2080.APS.05526.

Kuun, H. & Borchert, A. 2002. Regulation of enzymatic lipid peroxidation: the interplay of peroxidizing and peroxide reducing enzymes. *Free Radical Biological Medicine.* 33: 154-172.

Levesque, P. G. 2008. Use of 1-methylcyclopropene and hydrogen peroxide to study apple ripening physiology. Washington State University. *Department of Horticulture and Landscape Architecture.* Pp. 59-66.

Lubran, M. M. 1978. The measurement of total serum proteins by the Biuret method. *Animal Clinical Laboratory Science.* 8: 106-110.

Meluzzi, A., Primiceri, G., Giordani, R., & Fabris, G. 1992. Determination of blood constituents reference values in broilers. *Poultry Science.* 71: 337-345.

Merck Manual 2012. Haematological reference ranges. Mareck verterinary manual. Received from http://www.merckmanuals.com/.

Mirghaed, A. T., Hoseini, S. M., & Ghelichpour, M. 2018. Effects of dietary 1, 8-cineole supplementation and physiological, immunological and antioxidant responses to crowding stress in rainbow trout (Oncorhynchus mykiss). *Fish and Shellfish Immunology.* 81:182-188.

Mirghaed, A. T., Fayaz, S., & Hoseini, S. M. 2019. Effects of dietary 1,8-cineole supplementation on serum stress and antioxidant markers of common carp (*Cyprinus carpio)* acutely exposed to ambient ammonia. *Aquacculture.*15:8-15. https://doi.org/10.1016/j.aquaculture.2019.04.071.

Oberley, L. W., Leuthasuar, S. W. C., Oberley, T. D., & Loven, D. P. 1998. Lowered Superoxide Dismutase activity in distant organs of tumour bearing mice. *Journal of the National Cancer Institute.* 72: 1065-1074.

Odetola, O. M., Adejinmi, O. O., Owosibo, O. A., Banjo, O. T., & Awodola-Peters, O. O. 2019. Growth response, serum biochemistry and organ histopathology of broiler fed diets supplemented with graded levels of *Petiveria alliacea* root meal. *Internationl Journal of Poultry Science.* 18: 45-50. https://doi.org/10.3923/ijps.2019.45.50.

Oguntoye, M. A., Idowu, O. M. O., Sogunle, O. M., Akintunde, A. R., & Danladi, Y. 2018. Haematology and serum chemistry of finisher broiler chickens fed maize-cassava diet supplemented with methionine and inorganic sulphur. *Nigerian Journal of Animal Science.* 20(1): 152-162.

Okereke, C. O., Okereke, I. H., & Akinmutimi, A. H. 2019. Growth performance, haematological and serum biochemistry of broiler chickens fed Taro cocoyam *(Colocacia esculenta)* peel meal as feed ingredient. *Nigeria Journal of Animal Science.* 21(3): 334-341.

Okorie, K. C., Nkwocha, G. A., &Ndubuisi, E. C. 2011. Implications of feeding varying dietary levels of cassava leaf meal on finisher broiler: Performance, carcass, haematological and serological profiles. *Global Research Journal of Science.* 1: 58-66, ISBN: 2276-8300.

Olomieja, A. O., Olanrewaju, I. O., Ayo-Ajayi, J. I., Jolayemi, G. E., Daniel, U. O., & Mordi, R. C. 2021. Antimicrobial and

antioxidant properties of *Petiveria alliacea.* 4th International Conference on Science and Sustainable Development. Series: *Earth and Environmental Science*. 655:012025. Doi:10.1088/1755-1315/655/1/012015.

Oni, O. O., Alabi, J. O., Adewole, M. A., & Adegbenjo, A. A. 2018. Effect of phytobiotics (mixture of garlic, ginger and chaya leaf) on growth performance, haematological and biochemical indices of pullet chicks. *Slovak Journal of Animal Science.* 51(2): 69-78.

Owosibo, A. O., Odetola, O. M., Odunsi, O. O., Adejinmi, O. O., & Lawrence-Azua, O. O. 2013. Growth, haematological and serum biochemistry of broilers fed probiotics-based diets. *African Journal of Agriculture.* 8(41): 5076-5081.

Oyeleke, A., Adeyemi, O., Egbeyale, L., Sobayo, R., Obasa, O., & Okukenu, O. 2021. Growth performance, blood indices and intestinal organ development of pullet chicks administered aqueous extracts of guinea hen weed (*Petiveria alliacea*). *Slovak Journal of Animal Science.* 54(1): 21-32. ISSN 1337-9984.

Pandey, K. B., & Rizvi, S.I. 2011. Biomarkers of oxidative stress in red blood cells. *Biomedicine.* 155(2):131-136. Doi: 10.5507/bp.2011.027.

Raintree Nutrition. 2013. Presence of compounds in anamu (*Petiveria alliacea)*. http://www.rain-tree.com/anamu-chemicals.pdf.

Reddy, A. R., Chaitanya, K. V., & Vivekanandan, M. 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of Plant Physiology.* 161: 1189-1202.

Reitman, S., & Frankel, S. 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Animal Journal of Clinical Pathology.* 28(1): 56-63.

Ritchie, B. W., Harrison, J. G., & Harrison, R. L. 1994. Avian medicine. Winger's Publishing, Inc. Florida. Pp. 176-198.

Roos, G., & Messens, J. 2011. Protein sulfenic acid formation: from cellular damage to redox regulation. *Free Radical Biology and Medicine.* 15(2): 314-326. https://doi.org/10.1016/j.freeadbiomed.2011.04.031.

SAS 2009. Statistical Analysis System Institute, North Carolina USA. V.9.2.

Shtukmaster, S., Ljubuncic, P., & Bomzon, A. 2010. The effect of an aqueous extract of *Teucrium polium* on glutathione homeostasis *In vitro:* A possible mechanism of its hepatoprotectant action. *Advances in Pharmacological Science.* https://doi.org/10.1155/2010/938324.

Siems, W.G., Sommerburg, O., & Grune, T. 2000. Erythrocyte free radical and energy metabolism. *Clinical Neprology.*53:S9-S17.

Siu, G. M., & Draper, H. H., 1978. A survey of the malonaldehyde content of retail meats and fish. *Journal of Food Science*. 43(4): 1147-1149. https://doi.org/10.1111/j.1365-2621.1978.tb15256.x.

Soares, B. O., Oliveria, M. B. N., Mansur, E., Dantas, F. J. S., De Mattos, J. C. P., Caldeira-de-Araujo, A., & Gagilardi, R. F. 2014. Effect of extracts from field and *in vitro* plants of *Petiveria alliacea* L. on plasmidial DNA. *Journal of Medicinal Plant Research.* 8(33): 1101-1109. https://doi.org/10.5897/JMPR2014.5471.

Sobayo, R. A., Okonkwo, I. J., Sanwo, K. A., Muhammad, S. B., Oso, O. A., Eruvbetine, D., & Oguntona, E. B. 2018. Effect of natural plant; guinea hen weed (*Petiveria alliacea*) parts on growth and carcass indices of finishing broiler chickens. *Nigerian Agricultural Journal.* 49(2): 152-160.

Soomro, S. 2019. Oxidative stress and inflammation. *Open Journal of Immunology.* 9:1-20. https://doi.org/10.4236/oji.2019.91001.

Suliaman, A. F., Kazeem, M. O., Waheed, A. M., Temowo, S. O., Azeez, I. O., Zubair, F. I., Adeyemi, T. A., Nyang, A., & Adeyemi, O. S. 2014. Antimicrobial and toxic potentials of the aqueous extracts of *Allium sativun, Hibiscus sabdariffa* and *Zingiber officinale* in Wistar rats. *Journal*

of Taibah University for Science. 8: 315-322.

Thrall, M. A., & Weiser, M. G. 2002. Haematology. In: Hendrix CM. Laboratory procedures for veterinary technician's 4th Edition. Mosby Inc. Missouri 29-74.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., & Telser, J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry and Cell Biology.* 39:44-48. https://doi.org/10.1016/j.biocel.2006.07.001.

Varley, H., Gowenlock, A. H., & Bell, M. 1980. The plasma protein In: practical Clinical Biochemistry, Vol 15th Edition. William Heineamann Medical Books Ltd, London, pp 535-595.

Velayutham, R., Sankaradoss, N., & Ahamad, K. F. 2012. Protective effect of tannins from *Ficus racemose* in hypercholestererolemia and diabetes induced vascular damage in rats. *Asian Pacific Journal of Tropical Medicine.* 5: 367-73.

Xianyong, Ma., Deng, Dun., Chen, & Weidong. 2017. Inhibitors and activators of SOD, GSH-Px, and CAT. *Intechopen.* Doi:10.5772/65936.

Yasunari, K., Maeda, K., Nakamura, M., & Yoshikawa, J. 2002. Oxidative stress in leukocytes is a possible link between blood pressure, blood glucose, and C-reacting protein. *Journal of the American Heart Association.* 39:777-780.

Yousef, M. I., Saad, M. I., & El-Shennawy, I. K. 2009. Protective effect of grape seed proanthocyandin extract against oxidative stress induced by cisplatin in rats. *Food Chemistry and Toxicology.* 46: 1176-1183. https://doi.org/10.1016/j.fet.2009.02.007.

Yusuf, A. O., Surajudeen, M. B., & Akinyemi, B. T. 2023. Common plant bioactive components adopted in combating gastrointestinal nematodes in small ruminant- A review. *Agricultura Scientia.* 20(1):61-73. https://doi.org/10.18690/agricsci.20.1.7.

Zhang, Y., Usman, S., Li, Q., Li, F., Zhang, X., Nussio, L.G., & Guo, X. 2024. Effects of antioxidant-rich *Lactiplantibacillus plantarum* inoculated alfalfa silage on rumen fermentation, antioxidant and immunity status, and mammary gland gene expression in diary goats. *Journal of Animal Science and Biotechnology.* 15:9. https://doi.org/10.1186/s40104-023-00977-3.

Date received: 26th August, 2025

Date accepted: 15th December, 2025