

**THE INFLUENCE OF ANTIOXIDANT FROM *Aspilia africana* AQUEOUS  
LEAF EXTRACT ON HAEMATOLOGICAL PERFORMANCE OF ROSS 308  
BROILER CHICKENS**

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FACULTY OF AGRICULTURE  
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BENIN CITY, EDO STATE, NIGERIA.**

**NOVEMBER, 2025**

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**A PROJECT SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE  
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**NOVEMBER, 2025**

## **CERTIFICATION**

This is to certify that this project was carried out by AKINPETIDE Oluwabunmi Blessing (Miss), Matriculation Number, AGR2004294 under the guidance of the project supervisors approved by the Department of Animal Science, University of Benin, Benin city, Nigeria.

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**PROF. J. A. IMASUEN**  
**PROJECT SUPERVISOR**

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**DATE**

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**DR. N.C. AKAEZE**  
**HEAD OF DEPARTMENT**

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**DATE**

## **DEDICATION**

This project is dedicated to God Almighty, whose grace, wisdom, and strength sustained me from the beginning of this work to the end.

To my mother, who have been my greatest source of encouragement, her sacrifices, support and prayers have seen me through my academic pursuit.

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## ABSTRACT

This study investigated the influence of *Aspilia africana* aqueous leaf extract on the haematological performance of ROSS 308 broiler chickens. This experiment was conducted at the Poultry Unit of the Uniben Farm Project, Animal science Department, University of Benin. A total of one hundred and twelve (112) day-old broiler chicks were used for the experiment which lasted seven (7) weeks. The birds were randomly distributed into four (4) treatments groups (T1, T2, T3, and T4) and replicated twice in a complete randomized design, which each treatment having 14 birds. T1 served as control and received clean water without the extract, while T2, T3, and T4 received *Aspilia africana* aqueous leaf extract at concentrations of 100 ml, 150 ml, and 200 ml per 7 litre of drinking water respectively. Fresh leaves of *Aspilia africana* were collected, washed, air-dried overnight, milled, and soaked in boiled water to prepare the aqueous extract, the extract was kept under 2°C in a refrigerator to preserve for use and these processes were maintained every 4 days to maintain freshness. Standard management practices, including vaccination, biosecurity, and routine monitoring, were observed throughout the study. At the end of the feeding trial, blood samples were collected via the jugular vein for haematological analysis. Parameters evaluated included Packed Cell Volume (PCV), Haemoglobin concentration (Hb), Red Blood Cell (RBC) count, White Blood Cell (WBC) count, and Platelet count. Data obtained were subjected to Analysis of Variance (ANOVA), and significant means were separated using Duncan's Multiple Range Test at a probability level of  $p < 0.05$ . Results showed that the administration of *Aspilia africana* extract had a notable effect on haematological indices. Birds receiving 150 ml and 200 ml of the extract exhibited improved PCV, Hb and RBC. The WBC and Platelet was lowered in 200 ml group as compared to the control group. The enhancements observed suggest increased erythropoiesis, stronger immune response, and better physiological stability in treated birds. In conclusion, *Aspilia africana* aqueous leaf extract enhanced the haematological performance of ROSS 308 broilers in a dose-dependent manner. The extract can serve as a natural antioxidant and haematinic additive in broiler production, offering a low-cost and readily available alternative to synthetic supplements; and may be recommended for use by farmers.

# CHAPTER ONE

## INTRODUCTION

### 1.1 Background of the Study

Poultry production is a rapidly expanding sector of the livestock industry worldwide, providing a major source of high-quality animal protein in the form of meat and eggs (Adeniji *et al.*, 2022). The broiler chicken, particularly the ROSS 308 strain, is one of the

most popular commercial breeds known for its fast growth rate, efficient feed conversion ratio, and high-quality carcass yield (Onunkwo *et al.*, 2020). However, the productivity of broiler chickens is often influenced by nutritional, environmental, and physiological factors that affect their growth performance, immune response, and overall health (Oke *et al.*, 2021).

In recent years, there has been increasing concern about the excessive use of synthetic growth promoters, antibiotics, and feed additives in poultry diets. Although these substances enhance growth and disease resistance, they pose potential risks such as drug residues in meat, antibiotic resistance, and high production costs (Ahiwe *et al.*, 2021). This has prompted the search for natural, safe, and effective alternatives particularly from medicinal plants known for their antioxidant, antimicrobial, and immunomodulatory properties (Adeniran and Lawal, 2019).

One promising plant in this regard is *Aspilia africana* (Compositae), commonly known as “Hemorrhage plant” or “Wild sunflower.” It is a perennial herb widely

distributed across tropical Africa and traditionally used for wound healing, blood clotting, and inflammation control (Edeoga and Okoli, 2018). Phytochemical studies have revealed that *Aspilia africana* contains flavonoids, tannins, saponins, alkaloids, and phenolic compounds all of which exhibit strong antioxidant and therapeutic properties (Ezeonu *et al.*, 2020).

Antioxidants play a critical role in reducing oxidative stress in poultry by scavenging free radicals that can damage cells, impair immune function, and reduce growth performance (Surai *et al.*, 2019). In fast-growing broilers such as ROSS 308, oxidative stress is a common physiological challenge due to high metabolic activity and rapid muscle growth. Therefore, supplementation with natural antioxidants like *Aspilia africana* extract could improve blood quality, boost immune response, and enhance overall performance (Adebayo *et al.*, 2022).

Haematological parameters such as packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC), and platelet count (PLT) are key indicators of the physiological and health status of poultry birds (Etim *et al.*, 2020). They reflect the oxygen-carrying capacity of the blood, immune competence, and the overall well-being of the animal. Variations in these parameters can be used to assess the effect of dietary treatments and stress conditions (Olawuwo *et al.*, 2019).

Thus, evaluating the influence of *Aspilia africana* aqueous leaf extract on the haematological performance of broiler chickens provides insight into its physiological impact and potential as a natural antioxidant additive.

## **1.2 Statement of the Problem**

Despite the promising growth of poultry production in Nigeria, the industry still faces significant challenges related to poor health management, oxidative stress, and high mortality rates due to infections and nutritional deficiencies (Esonu *et al.*, 2020). The overreliance on synthetic antibiotics as growth promoters has led to resistance problems, reduced consumer confidence, and export restrictions due to drug residues in poultry products (Ojediran *et al.*, 2019).

There is, therefore, a pressing need for safe, affordable, and effective natural alternatives that can enhance broiler productivity without compromising product safety or consumer health. *Aspilia africana* is a readily available medicinal plant that has been traditionally used for its antioxidant and blood-boosting properties. However, scientific data on its haematological effects in modern commercial broilers like ROSS 308 remain limited.

This research, therefore, seeks to determine the influence of antioxidants from *Aspilia africana* aqueous leaf extract on the haematological performance of ROSS 308 broiler chickens.

## **1.3 Justification of the Study**

The search for herbal alternatives to synthetic feed additives aligns with the global trend toward organic and antibiotic-free poultry production (FAO, 2021). *Aspilia africana* offers a cheap, locally available source of phytochemicals with potential antioxidant and haemopoietic benefits. Studying its effect on blood parameters in broilers will help to:

1. Demonstrate its potential to improve blood oxygenation and immunity.
2. Provide scientific justification for its inclusion in poultry diets.
3. Reduce dependency on costly synthetic drugs.
4. Promote sustainable and residue-free poultry production in Nigeria.

Moreover, the findings of this study will contribute to the growing body of knowledge on ethnoveterinary medicine and may open new opportunities for commercial herbal feed formulations for livestock farmers.

#### **1.4 Scope of the Study**

This study was designed to evaluate the haematological responses of ROSS 308 broiler chickens administered varying concentrations of *Aspilia africana* aqueous leaf extract under controlled experimental conditions. The scope of the study specifically covers the effects of *Aspilia africana* aqueous leaf extract on selected blood parameters that serve as important indicators of physiological and health status in poultry. These parameters include Packed Cell Volume (PCV), Haemoglobin concentration (Hb), White Blood Cell count (WBC), Red Blood Cell count (RBC), and Platelet count (PLT). Each of these indices provides critical information about

oxygen-carrying capacity, immune response, metabolic activity, and overall well-being of the birds.

## **1.5 Objectives of the Study**

### **1.5.1 General Objective**

To evaluate the influence of antioxidants from *Aspilia africana* aqueous leaf extract on the haematological performance of ROSS 308 broiler chickens.

### **1.5.2 Specific Objectives**

The specific objectives of this study are to:

1. determine the effects of *Aspilia africana* aqueous extract on the packed cell volume (PCV), hemoglobin concentration (Hb), and red blood cell (RBC) counts of broiler chickens.
2. evaluate the influence of *Aspilia africana* extract on the white blood cell (WBC) and platelet counts.
3. assess the dose-dependent antioxidant impact of 100 ml, 150 ml, and 200 ml aqueous leaf extract on blood parameters of ROSS 308 broilers.
4. compare the hematological responses of treated birds with the control group.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 The Poultry Industry and Broiler Chicken Production

The poultry industry remains one of the most dynamic and rapidly expanding subsectors of the livestock industry in Nigeria. It plays a crucial role in addressing the nation's food security challenges by providing affordable and high-quality animal protein in the form of meat and eggs. Poultry farming also serves as a major source of livelihood and income generation for millions of Nigerians, ranging from smallholder farmers operating backyard flocks to large-scale commercial producers supplying urban markets. According to Ahiwe *et al.* (2021), poultry production has become an essential component of sustainable agricultural development because of its short production cycle, efficient resource use, and quick turnover rate compared to other livestock species.

Among all classes of poultry, the broiler chicken (*Gallus gallus domesticus*) occupies a prominent position in meat production due to its remarkable growth potential, excellent feed conversion efficiency, and the ability to reach market weight within a relatively short period, usually six to eight weeks. Broilers have been selectively bred

for rapid growth, early maturity, and high carcass yield, which makes them economically attractive to both commercial and small-scale producers. The ROSS 308 strain, in particular, is one of the most widely reared commercial broiler breeds across the world. It has been genetically improved for optimal growth rate, superior breast meat yield, efficient feed utilization, and adaptability to a wide range of environmental and management conditions (Onunkwo *et al.*, 2020).

Despite these genetic advancements, broiler production in Nigeria continues to face numerous challenges that limit optimal performance. These include nutritional stress, environmental fluctuations, poor management practices, and disease outbreaks, all of which can induce physiological stress and oxidative imbalance in birds. Oxidative stress arises when the production of reactive oxygen species (ROS) exceeds the bird's antioxidant defense capacity, resulting in cellular damage, suppressed immunity, and reduced productivity (Surai *et al.*, 2019). Such stress factors not only compromise growth rate and feed efficiency but also deteriorate meat quality and shelf life, leading to significant economic losses.

To counteract these negative effects, poultry scientists and nutritionists have increasingly explored the use of natural feed additives with antioxidant, antimicrobial, and immune-boosting properties. These functional additives, such as phytobiotics, probiotics, organic acids, and medicinal plant extracts, help to enhance gut health, improve nutrient absorption, and strengthen the immune system of the birds. Among

them, plant-derived antioxidants have attracted special interest due to their safety, cost-effectiveness, and ability to neutralize free radicals naturally (Khan *et al.*, 2020).

The incorporation of medicinal plants like *Aspilia africana* into broiler feeding regimes reflects a growing shift toward sustainable and eco-friendly poultry production practices. Such herbal supplements are being studied not only for their potential to improve growth performance but also for their capacity to enhance blood quality, immunity, and oxidative stability in poultry. In the context of developing countries like Nigeria, where the cost of conventional synthetic additives can be prohibitive, the exploration of indigenous plants as functional feed ingredients provides an affordable alternative for maintaining bird health and ensuring profitable production.

Overall, broiler chicken production forms a vital link between agricultural advancement and nutritional well-being in Nigeria. Continuous research on natural performance enhancers such as *Aspilia africana* is therefore essential for promoting productivity, ensuring food safety, and sustaining the growth of the poultry industry under tropical production conditions

## **2.2 Oxidative Stress and the Role of Antioxidants in Poultry**

In poultry production, maintaining a balance between metabolic activity and oxidative stability is essential for sustaining growth, health, and productivity. Oxidative stress occurs when the generation of reactive oxygen species (ROS) exceeds the capacity of the bird's natural antioxidant defense system to neutralize them (Surai *et al.*, 2018).

Under normal physiological conditions, ROS play useful roles in cellular signaling and immune defense. However, excessive accumulation leads to oxidative damage that can compromise vital biological functions.

Broiler chickens are particularly prone to oxidative stress because of their fast growth rate, high metabolic demand, and sensitivity to environmental stressors such as heat, overcrowding, nutrient imbalances, and disease challenges (Iqbal *et al.*, 2020). These stressors increase oxygen consumption and electron leakage in the mitochondria, resulting in elevated production of free radicals. When left uncontrolled, ROS attack lipids, proteins, and DNA, leading to lipid peroxidation, enzyme inactivation, cell membrane disruption, and ultimately, a decline in feed efficiency, immune competence, and overall performance (Rehman *et al.*, 2018).

To counteract these effects, the body relies on antioxidant systems that either prevent the formation of free radicals or neutralize them once produced. These antioxidants can be classified into endogenous (naturally occurring) and exogenous (dietary) categories. Endogenous antioxidants include key enzymatic defenses such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which act in coordination to scavenge and convert reactive molecules into harmless by-products like water and oxygen. Exogenous antioxidants, on the other hand, are obtained from the diet and include compounds such as vitamin C, vitamin E, selenium, zinc, and various plant-derived phytochemicals (Adegbeye *et al.*, 2020).

In recent years, attention has shifted toward the use of natural antioxidants derived from medicinal plants as a sustainable and health-promoting alternative to synthetic ones. Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), though effective, have raised safety concerns due to their potential toxicity and carcinogenic effects when consumed over time (Zhang *et al.*, 2023). In contrast, phytogetic antioxidants, which include flavonoids, phenolic acids, tannins, saponins, and alkaloids, are naturally occurring plant compounds with strong radical-scavenging and metal-chelating properties. These bioactive substances enhance antioxidant enzyme activity, improve immune response, and protect cellular integrity against oxidative injury.

Among the promising medicinal plants explored in tropical regions, *Aspilia africana* stands out for its remarkable antioxidant and haemopoietic potential. The plant is rich in phytochemicals such as flavonoids, tannins, saponins, terpenoids, and alkaloids, which collectively contribute to its ability to reduce oxidative stress, stimulate blood formation, and promote tissue repair. When included in poultry feeding regimes, extracts from *Aspilia africana* may help mitigate the negative effects of oxidative stress, enhance red blood cell production, and improve the overall health status of broiler chickens.

Understanding oxidative stress and the mechanisms by which antioxidants function is therefore fundamental to modern poultry nutrition. By incorporating safe, natural antioxidant sources such as *Aspilia africana*, poultry producers can enhance

productivity, improve bird welfare, and ensure safer, chemical-free meat production for consumers.

### **2.3 Botanical Description and Distribution of *Aspilia africana***

*Aspilia africana* (Pers.) C.D. Adams belongs to the family Asteraceae (Compositae). It is a perennial herb commonly known as “Wild sunflower” or “Haemorrhage plant.” The plant is native to tropical Africa and widely distributed across Nigeria, Ghana, Cameroon, and Kenya (Edeoga and Okoli, 2018). It typically grows along roadsides, farmlands, and open grasslands, reaching heights of 60–120 cm with erect, hairy stems and bright yellow-orange flowers.

In traditional medicine, *A. africana* is used for wound healing, to stop bleeding, and as an anti-inflammatory agent (Ezeonu *et al.*, 2020). The leaves are the most commonly used part, either in crude or aqueous extract form, due to their rich content of bioactive compounds.

#### **2.3.1 Phytochemical Constituents of *Aspilia africana***

Phytochemical screening of *Aspilia africana* leaves has shown that the plant is rich in a wide range of bioactive compounds that contribute to its medicinal and nutritional value. Several studies have identified the presence of flavonoids, tannins, alkaloids, saponins, terpenoids, glycosides, and phenolic compounds (Ajayi *et al.*, 2020). These naturally occurring compounds are responsible for many of the plant’s pharmacological activities, including its antioxidant, antimicrobial, anti-inflammatory, haemostatic, and wound-healing properties (Ezeonu *et al.*, 2020).

Each of these phytochemicals plays a distinct but complementary role in promoting health and improving physiological performance in animals:

1. Flavonoids: These are powerful natural antioxidants capable of neutralizing reactive oxygen species (ROS) before they cause cellular damage. Flavonoids stabilize free radicals by donating hydrogen atoms or electrons, thereby preventing lipid peroxidation and protecting cell membranes especially those of red blood cells from oxidative injury. This mechanism not only enhances blood stability but also supports immune function and tissue repair (Oboh *et al.*, 2019).
2. Tannins: Tannins are known for their astringent and antimicrobial properties. They help to strengthen the gut lining, reduce intestinal permeability, and limit the growth of harmful microorganisms in the digestive tract. By improving gut integrity, tannins indirectly enhance nutrient absorption and feed efficiency in poultry. Their ability to bind with bacterial proteins also contributes to a healthier gastrointestinal environment (Ademola and Eloff, 2018).
3. Saponins: These compounds are glycosides that possess both surface-active and physiological properties. In poultry nutrition, saponins play a role in cholesterol metabolism, helping to lower serum cholesterol levels by binding to bile acids. They also exhibit immune-modulatory effects, enhancing the body's defense mechanisms against infections and stress (Adebayo *et al.*, 2022).

4. Phenolic Compounds: Phenolics are among the most potent plant-based antioxidants. They act by donating hydrogen atoms to stabilize free radicals and by chelating metal ions that catalyze oxidative reactions. Their activity contributes significantly to maintaining oxidative stability in biological systems and supports the antioxidant enzyme network in the body (Nworgu *et al.*, 2021).
5. Terpenoids and Alkaloids: Though present in smaller quantities, these compounds contribute additional health benefits. Terpenoids exhibit antimicrobial and anti-inflammatory properties, while alkaloids possess diverse pharmacological actions, including analgesic and antipyretic effects. Together, they enhance the plant's therapeutic efficacy and broaden its biological applications.

The combined action of these phytochemicals gives *Aspilia africana* its remarkable health-promoting potential. The antioxidant activity of the plant, in particular, is largely attributed to its high phenolic and flavonoid content, which work synergistically to reduce oxidative stress, enhance blood quality, and improve overall physiological performance in animals supplemented with its extract.

Thus, when incorporated into broiler feeding programs, *Aspilia africana* may serve as a natural source of antioxidants that promote better health, improved growth performance, and greater resistance to oxidative challenges offering a sustainable alternative to synthetic additives in poultry production.

### **2.3.2 Mechanism of Antioxidant Action of *Aspilia africana***

The antioxidant activity of *Aspilia africana* is derived from the synergistic action of its diverse phytochemical constituents, particularly flavonoids, phenolic acids, tannins, and saponins. These bioactive compounds function through multiple interrelated mechanisms that help protect body cells from oxidative injury, enhance immune competence, and sustain overall metabolic balance in poultry.

#### **1. Free Radical Scavenging**

One of the most important antioxidant mechanisms of *Aspilia africana* is its ability to neutralize free radicals. The polyphenolic compounds present in the plant donate hydrogen atoms or electrons to unstable molecules known as reactive oxygen species (ROS), thereby converting them into more stable and less harmful forms. This reaction prevents the cascade of oxidative damage that could otherwise lead to the destruction of vital biomolecules such as lipids, proteins, and DNA. By scavenging free radicals, *Aspilia africana* helps maintain the structural and functional integrity of body cells, especially those of rapidly dividing tissues like blood cells (Oboh *et al.*, 2019).

#### **2. Metal Chelation**

Transition metals such as iron ( $\text{Fe}^{2+}$ ) and copper ( $\text{Cu}^{2+}$ ) can catalyze the generation of free radicals through Fenton-type reactions, thereby accelerating oxidative stress. The tannins and flavonoids found in *Aspilia africana* possess strong metal-chelating properties, meaning they bind to these metal ions and

render them inactive. This prevents the metals from participating in harmful oxidative reactions that damage cellular components. By limiting metal-induced oxidation, the plant extract effectively reduces oxidative pressure within tissues and maintains redox homeostasis (Kumar *et al.*, 2020).

### 3. **Enzyme Modulation**

Another key mechanism involves the enhancement of the body's endogenous antioxidant enzyme systems. *Aspilia africana* extract has been shown to upregulate the activity of enzymes such as catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD), all of which play critical roles in detoxifying reactive oxygen species. SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide, which is then broken down by CAT and GPx into water and oxygen. This enzymatic defense system prevents oxidative build-up and promotes efficient cellular metabolism, especially under stressful production conditions in broilers (Oboh *et al.*, 2019).

### 4. **Inhibition of Lipid Peroxidation**

Lipid peroxidation refers to the oxidative degradation of lipids within cell membranes, a process that compromises membrane integrity and fluidity. The antioxidant compounds in *Aspilia africana*, particularly flavonoids and phenolics act by intercepting free radicals before they attack membrane lipids. This preserves the structure of erythrocyte membranes and prevents

haemolysis, thereby supporting red blood cell stability and efficient oxygen transport. By minimizing lipid peroxidation, the extract also enhances tissue resilience, meat quality, and overall bird performance (Adebayo *et al.*, 2022).

Through these combined pathways, *Aspilia africana* extract strengthens the bird's antioxidant defense system, supports immune function, and maintains normal metabolic processes even under stress conditions. Its multifunctional antioxidant mechanism makes it a promising natural supplement for improving health, performance, and oxidative balance in poultry production.

#### **2.4 Haematological Responses as Indicators of Health in Poultry**

Haematological parameters are valuable diagnostic tools used to assess the physiological and health status of birds. They reflect how the body responds to nutrition, disease challenges, environmental stress, and the general management system. In poultry science, the evaluation of blood parameters is essential not only for understanding the internal state of the animal but also for predicting its growth performance, productivity, and immune competence (Etim *et al.*, 2018).

Blood, being the primary transport medium in the body, plays a crucial role in distributing nutrients, oxygen, hormones, and metabolic wastes. Any alteration in its composition can therefore indicate underlying physiological disturbances. The haematological profile of a bird can reveal early signs of stress, infection, anemia, or nutritional deficiencies long before external symptoms become visible. As such, it

serves as a reliable biomarker for monitoring the effects of feed additives, environmental factors, and health interventions in poultry production systems (Adebayo *et al.*, 2022).

Among the most important haematological indices are the Packed Cell Volume (PCV), Haemoglobin concentration (Hb), Red Blood Cell count (RBC), White Blood Cell count (WBC), and Platelet count (PLT). Each of these parameters provides specific insight into the bird's physiological condition:

1. **Packed Cell Volume (PCV):** This represents the proportion of red blood cells in the total blood volume and is an indicator of the bird's oxygen-carrying capacity. A higher PCV suggests improved erythropoiesis (red blood cell production) and better tissue oxygenation, while a lower PCV may indicate anemia, dehydration, or poor nutritional status (Oladokun *et al.*, 2019).
2. **Haemoglobin (Hb):** Haemoglobin is the iron-containing protein in red blood cells responsible for transporting oxygen from the lungs to body tissues. Adequate haemoglobin levels are necessary for efficient aerobic metabolism and energy production. A reduction in Hb concentration may result from poor dietary iron or chronic infections, whereas an increase often reflects enhanced oxygen delivery and general vitality (Ihedioha *et al.*, 2016).
3. **Red Blood Cell Count (RBC):** This measures the number of erythrocytes per microliter of blood. RBC count correlates with the bird's ability to deliver oxygen to tissues, sustain energy metabolism, and support rapid growth.

Factors such as antioxidants and phytogetic compounds can stimulate erythropoiesis, resulting in increased RBC levels (Olawumi *et al.*, 2020).

4. **White Blood Cell Count (WBC):** WBCs are central to the immune system, providing defense against infections and inflammation. Elevated WBC counts generally indicate strong immune stimulation or an active response to infection, while low counts may reflect immunosuppression. Phytochemicals with immunomodulatory properties, such as those found in *Aspilia africana*, have been shown to improve WBC production and activity (Adeyemo *et al.*, 2021).
5. **Platelets (PLT):** Platelets play a key role in blood clotting and wound repair. Their levels serve as indicators of vascular integrity and blood coagulation efficiency. A healthy platelet count supports effective haemostasis and minimizes blood loss during injuries or stress conditions.

Haematological studies also help in evaluating the safety and physiological compatibility of herbal feed additives used in poultry diets. When an additive such as *Aspilia africana* does not negatively alter blood parameters, it implies that the extract is non-toxic and safe for use at the administered dosage levels. Moreover, improved haematological indices following supplementation often point to enhanced nutrient absorption, antioxidant protection, and better overall health performance in the birds (Esonu *et al.*, 2020).

In essence, haematological responses provide a window into the internal well-being of poultry. They reflect how the birds interact with their nutrition, environment, and

management conditions. Therefore, evaluating these parameters is a critical component in determining the physiological impact of *Aspilia africana* extract and other natural antioxidants in broiler production.

#### **2.4.1 Importance of Haematological Assessment in Animal Studies**

Haematological assessment is one of the most vital diagnostic tools used in animal science and veterinary research to evaluate the internal physiological condition of animals. It provides critical insight into the health status, nutritional adequacy, metabolic function, and general well-being of livestock under experimental or field conditions. In poultry production, blood analysis is indispensable for understanding how birds respond to changes in diet, management, or environmental stress. This is because blood serves as a sensitive indicator that reflects alterations in the physiological and biochemical balance of the body (Etim *et al.*, 2018).

Essentially, the blood system functions as a dynamic mirror of the animal's health. Every nutrient absorbed, drug administered, or environmental stress experienced by the bird leaves a measurable imprint on the blood profile. When birds are exposed to nutritional supplements or herbal extracts—such as *Aspilia africana*—the haematological profile helps determine whether these interventions are beneficial, neutral, or harmful. In this sense, blood analysis becomes a window into the internal adaptations taking place within the animal's body in response to external inputs (Adebayo *et al.*, 2022).

Haematological tests are particularly important in experimental research because they allow for the early detection of subclinical conditions that may not yet manifest as visible symptoms. Parameters such as Packed Cell Volume (PCV), Haemoglobin concentration (Hb), and Red Blood Cell count (RBC) reveal the oxygen-carrying capacity of the blood and the efficiency of erythropoiesis (red blood cell production). Similarly, White Blood Cell count (WBC) and Platelet count (PLT) indicate the state of immune function and blood clotting ability respectively (Ihedioha *et al.*, 2016). Monitoring these parameters provides a reliable picture of how the animal's body maintains homeostasis, defends itself against pathogens, and responds to dietary interventions.

In poultry nutrition studies, haematological evaluations also serve as an important bioassay for the safety of feed additives. When new feed ingredients or plant extracts are introduced into the diet, they can influence blood composition either positively or negatively. A significant improvement in haematological parameters suggests enhanced physiological efficiency, while abnormal values may indicate toxic or stressful effects on the bird's system. Thus, blood indices are routinely used to evaluate the haematinic potential, immune-modulating properties, and possible toxicity of experimental feed materials (Esonu *et al.*, 2020).

Beyond serving as diagnostic indicators, haematological data are also valuable for interpreting metabolic efficiency and production potential. Birds with optimal blood profiles often exhibit better feed conversion ratios, faster weight gain, and stronger

immunity compared to those with depressed values. This correlation has led to the increasing use of haematological parameters as biomarkers for evaluating the effectiveness of antioxidants and herbal additives such as *Aspilia africana*, which are known to improve erythrocyte stability and oxidative balance (Adeyemo *et al.*, 2021). Moreover, haematological studies contribute significantly to animal welfare assessment, particularly under tropical production conditions where birds are frequently exposed to environmental stressors such as heat, humidity, and pathogens. Evaluating blood parameters provides a scientific basis for determining the physiological cost of these stressors and identifying effective strategies nutritional or managerial that can mitigate their impact.

In research involving medicinal plants, haematological evaluations are particularly critical because they help distinguish between therapeutic efficacy and potential toxicity. For instance, a plant extract that improves haemoglobin levels and white blood cell counts without altering liver or kidney function indices can be considered safe for long-term use. Conversely, any extract that drastically lowers PCV, RBC, or Hb levels could signify haemolytic effects or suppressed erythropoiesis. This approach ensures that only safe, beneficial herbal additives are recommended for inclusion in animal diets (Ezeonu *et al.*, 2020).

In summary, haematological assessment in animal studies serves several interrelated purposes:

1. It provides an early indicator of disease, stress, or nutrient deficiency.

2. It helps evaluate the physiological impact and safety of experimental diets or treatments.
3. It aids in interpreting growth, metabolism, and immune response.
4. It supports decision-making in nutrition, breeding, and health management programs.

Given the increasing shift toward natural feed additives and plant-based antioxidants in modern poultry production, haematological evaluations are more relevant than ever. They help validate scientific claims about the efficacy of herbs like *Aspilia africana*, confirming whether these natural compounds truly enhance physiological performance and improve the overall health of broiler chickens without adverse side effects.

#### **2.4.2 Major Haematological Parameters and Their Physiological Roles**

Haematological parameters provide a scientific basis for assessing the health, nutritional, and physiological condition of poultry. They represent quantifiable indices that reflect the functionality of the circulatory and immune systems, as well as the body's ability to transport oxygen, fight infections, and maintain homeostasis (Etim *et al.*, 2018). Understanding the physiological significance of these parameters is crucial in evaluating how dietary treatments such as *Aspilia africana* supplementation affect the wellbeing and performance of broiler chickens.

The major haematological parameters commonly assessed in poultry research include Packed Cell Volume (PCV), Haemoglobin concentration (Hb), Red Blood Cell count (RBC), White Blood Cell count (WBC), and Platelet count (PLT). Each of these

indicators provides unique and complementary information about the internal state of the bird.

#### **2.4.2.1 Packed Cell Volume (PCV)**

Packed Cell Volume, also known as haematocrit, represents the proportion of blood occupied by red blood cells when centrifuged. It is expressed as a percentage and is a direct indicator of the oxygen-carrying capacity of the blood (Oladokun *et al.*, 2019).

A normal PCV value signifies balanced erythropoiesis and efficient oxygen delivery to tissues, which are essential for proper metabolic activity and growth.

Low PCV values typically indicate anaemia, which can result from nutritional deficiencies (especially iron, folate, or vitamin B<sub>12</sub>), parasitic infections, or oxidative stress. Conversely, elevated PCV values may reflect dehydration, excessive erythropoiesis, or an adaptive response to hypoxia. In poultry fed antioxidant-rich plants such as *Aspilia africana*, an optimal PCV often suggests that the extract enhances blood formation and protects red cells from oxidative damage, thereby promoting improved tissue oxygenation and vitality (Adeyemo *et al.*, 2021).

#### **2.4.2.2 Haemoglobin Concentration (Hb)**

Haemoglobin is an iron-containing protein found in erythrocytes that binds oxygen in the lungs and releases it to body tissues. The concentration of haemoglobin in the blood is a fundamental determinant of the bird's respiratory efficiency and metabolic capacity (Ihedioha *et al.*, 2016).

A decline in haemoglobin concentration may lead to hypoxia and reduced aerobic metabolism, which ultimately slows down growth rate and productivity. On the other hand, adequate haemoglobin levels reflect a robust oxygen transport system and healthy red blood cell formation. The haematinic (blood-building) properties of *Aspilia africana* have been linked to its rich content of iron and plant flavonoids, which enhance erythropoiesis and stabilize erythrocyte membranes. Birds receiving *Aspilia africana* supplementation often exhibit improved haemoglobin values, indicating better physiological efficiency and resilience to oxidative stress (Esonu *et al.*, 2020).

#### **2.4.2.3 Red Blood Cell Count (RBC)**

Red blood cells (erythrocytes) are the most abundant cells in the bloodstream, responsible for transporting oxygen and carbon dioxide between tissues and the lungs. The RBC count represents the number of erythrocytes per microlitre of blood and is closely associated with tissue oxygenation and energy metabolism (Etim *et al.*, 2018). An increase in RBC count is a positive indicator of enhanced oxygen supply, improved feed utilization, and better growth performance. In contrast, a reduced RBC count may signal anaemia, nutritional deficiency, or oxidative damage to erythrocytes. Supplementation with *Aspilia africana* has been shown to elevate RBC counts in broilers, likely due to the plant's antioxidant constituents that protect red blood cells from lipid peroxidation and promote erythropoietin activity (Ajayi *et al.*, 2020). This

helps maintain normal physiological functioning and ensures that metabolic demands during rapid growth are adequately met.

#### **2.4.2.4 White Blood Cell Count (WBC)**

White blood cells (leukocytes) are the body's primary line of defense against infectious agents, inflammation, and stress. The total WBC count reflects the bird's immune status and ability to respond to pathogenic challenges (Adebayo *et al.*, 2022).

High WBC counts often indicate an active immune response or mild infection, while low counts may suggest immunosuppression or chronic stress. The differential count, which includes lymphocytes, neutrophils, eosinophils, monocytes, and basophils, provides further insight into the type of immune activity occurring in the body. For instance, elevated lymphocyte counts are associated with enhanced adaptive immunity, whereas higher neutrophil levels may point to acute infections or inflammatory responses (Adeyemo *et al.*, 2021).

Several studies have reported that birds fed *Aspilia africana* extract show moderate increases in total and differential WBC counts, implying enhanced immune competence. The plant's bioactive compounds, such as flavonoids and phenolics, stimulate white cell proliferation and protect immune tissues from oxidative stress, thereby strengthening disease resistance and reducing mortality under production stress conditions (Ezeonu *et al.*, 2020).

#### **2.4.2.5 Platelet count (PLT)**

Platelets, or thrombocytes, are small, disk-shaped cell fragments that play a vital role in blood coagulation and tissue repair. The platelet count provides information about the haemostatic and vascular integrity of the bird (Etim *et al.*, 2018). Normal platelet levels ensure effective clot formation during injury, preventing excessive blood loss and supporting rapid healing.

A decrease in platelet count may indicate bone marrow suppression, haemorrhagic conditions, or toxicity, while an elevated count might be associated with inflammatory reactions or certain stress conditions. Birds receiving *Aspilia africana* supplementation often maintain normal platelet counts, reflecting the plant's safety and potential to promote vascular stability. The haemostatic properties of *A. africana*, widely recognized in traditional medicine, further underscore its role in controlling bleeding and supporting the body's natural healing processes (Oboh *et al.*, 2019)

### **2.4.3 Factors influencing haematological values in poultry**

Haematological parameters are dynamic indicators that vary with physiological, nutritional, environmental, and management conditions. The values obtained from blood analyses in poultry are not fixed; rather, they reflect the bird's adaptive response to both internal and external factors (Etim *et al.*, 2018). Understanding these influences is essential for interpreting blood test results accurately and for determining whether changes in haematological indices are due to experimental treatments, such as *Aspilia africana* supplementation, or other stress-related factors.

Several factors have been documented to affect the haematological profile of poultry, including nutrition, age, sex, health status, environmental temperature, stress, genetic strain, and management practices (Adebayo *et al.*, 2022). Each of these factors plays a distinctive role in shaping the blood characteristics of the bird.

#### **2.4.3.1 Nutritional Factors**

Nutrition is one of the most important determinants of blood composition. Adequate and balanced feeding ensures sufficient supply of essential nutrients required for haematopoiesis, oxygen transport, and immune defense. Deficiencies in proteins, iron, copper, vitamins (especially B-complex and vitamin E), and trace minerals can lead to anaemia, reduced haemoglobin synthesis, and lower packed cell volume (Ihedioha *et al.*, 2016).

Feed additives, including natural antioxidants and phytobiotics, also influence blood parameters by modulating nutrient absorption, metabolism, and antioxidant defense. Herbal supplements such as *Aspilia africana* have been shown to improve haematological indices by enhancing erythropoiesis and protecting red blood cells from oxidative damage. The plant's phytochemicals especially flavonoids and phenolics help stabilize erythrocyte membranes, boost iron utilization, and improve oxygen transport efficiency (Ajayi *et al.*, 2020).

#### **2.4.3.2 Age and Physiological Stage**

The age and developmental stage of poultry significantly affect their haematological values. Young chicks often exhibit lower PCV and haemoglobin levels due to an

immature haematopoietic system, while older birds tend to show higher erythrocyte counts and haemoglobin concentrations (Etim *et al.*, 2018). During rapid growth phases, the demand for oxygen and nutrient transport increases, leading to elevated red blood cell production. However, as birds approach maturity, these values may stabilize or slightly decline. Similarly, physiological conditions such as moulting, egg production, or stress from handling can cause transient fluctuations in blood indices.

#### **2.4.3.3 Sex and Genetic Strain**

Differences in sex and genetic makeup also influence blood profiles. Male broilers generally exhibit higher haemoglobin concentrations and packed cell volumes compared to females, possibly due to hormonal differences and greater metabolic activity (Adeyemo *et al.*, 2021). The genetic strain of the bird such as ROSS 308, Cobb 500, or Arbor Acres affects metabolism, feed efficiency, and physiological adaptation. Since haematological values are influenced by metabolic rate, strains selected for rapid growth and high feed conversion often display distinct blood characteristics reflecting their performance potential.

#### **2.4.3.4 Health Status and Disease Conditions**

The health condition of birds plays a major role in determining their haematological values. Infections, parasitic infestations, or inflammatory responses can cause alterations in blood indices. For instance, bacterial infections often lead to elevated white blood cell (WBC) counts due to immune activation, while parasitic infections

may cause a decline in red blood cell counts and haemoglobin levels, resulting in anaemia (Esonu *et al.*, 2020).

When birds are exposed to oxidative stress or toxins, such as aflatoxins or environmental pollutants, there is an increase in reactive oxygen species (ROS) which damage erythrocyte membranes. This condition leads to lower PCV, haemoglobin, and RBC values. Supplementation with *Aspilia africana* has been reported to counter these negative effects by improving antioxidant enzyme activity and enhancing immune function (Ezeonu *et al.*, 2020).

#### **2.4.3.5 Environmental Temperature and Stress**

Environmental conditions, especially temperature and humidity, exert profound effects on blood parameters. Under heat stress, poultry often experience dehydration, electrolyte imbalance, and oxidative stress, which can suppress immune function and alter blood values (Surai *et al.*, 2019). High ambient temperatures cause hemodilution, reducing PCV and haemoglobin concentrations, while cold stress may increase metabolic demand and RBC count as birds attempt to maintain body temperature.

The use of antioxidants like *Aspilia africana* helps mitigate these effects by reducing oxidative damage, stabilizing blood membranes, and improving the overall adaptability of birds to environmental challenges. The plant's bioactive compounds support the activity of antioxidant enzymes such as catalase and glutathione peroxidase, ensuring cellular protection during stressful conditions (Adegbeye *et al.*, 2020).

#### **2.4.3.6 Handling and Management Practices**

Improper handling, transport, or overcrowding can induce physiological stress, which affects blood homeostasis. Stress triggers the release of corticosteroids that can depress immune function, lower WBC counts, and alter glucose and haemoglobin levels (Etim *et al.*, 2018). Consistent handling or poor ventilation also increases the risk of hypoxia and oxidative imbalance, which may lower RBC and PCV values.

Good management practices such as maintaining optimal stocking density, providing clean water, adequate ventilation, and minimizing sudden changes in feed or environment are crucial for maintaining normal blood values. Under well-managed conditions, the introduction of herbal antioxidants like *Aspilia africana* tends to yield more consistent and positive haematological outcomes.

#### **2.4.3.7 Seasonal and Diurnal Variations**

Seasonal changes influence temperature, humidity, and feed quality, all of which affect the physiological responses of poultry. Birds raised during the wet season often show higher WBC counts and lower RBC values due to increased pathogen exposure and fluctuating environmental conditions. Diurnal variations differences between morning and evening blood collections can also affect parameters such as glucose, PCV, and WBC counts (Oladokun *et al.*, 2019). These variations must therefore be considered when interpreting haematological data in poultry research.

#### **2.4.4 Interpretation of blood indices in relation to health and nutrition**

Blood indices provide a scientific lens through which the overall health, metabolic state, and nutritional adequacy of poultry can be understood. In experimental studies, interpreting changes in these haematological parameters helps determine whether a dietary supplement or management intervention produces positive, neutral, or adverse physiological effects. Since blood acts as a transport medium for nutrients, gases, and metabolites, any alteration in its composition often reflects the body's adaptive responses to diet, environment, and disease (Etim *et al.*, 2018).

For broiler chickens, haematological interpretation is especially valuable because these birds are genetically predisposed to rapid growth and high metabolic activity. This makes their blood system highly sensitive to oxidative imbalance, nutritional deficiencies, and environmental stressors. Thus, evaluating how treatments such as *Aspilia africana* aqueous leaf extract affect blood indices provides direct insight into the extract's physiological impact, safety, and potential benefits as a natural antioxidant and haematinic agent.

##### **2.4.4.1 Packed Cell Volume (PCV) and Haemoglobin (Hb)**

The Packed Cell Volume (PCV) and Haemoglobin concentration (Hb) are closely related indicators that reflect the oxygen-carrying capacity of the blood. Higher PCV and Hb values generally suggest improved oxygen transport, efficient erythropoiesis, and enhanced metabolic activity (Oladokun *et al.*, 2019). In broilers, these values are vital because rapid tissue growth demands continuous oxygen supply.

A significant increase in PCV and Hb following dietary supplementation usually indicates that the feed or additive supports red blood cell synthesis and prevents haemolysis. For example, when broilers are administered *Aspilia africana* extract, elevated PCV and Hb values imply improved haematinic activity, likely due to the presence of iron, flavonoids, and other bioactive compounds that promote erythropoiesis and protect erythrocyte membranes from oxidative damage (Ajayi *et al.*, 2020).

Conversely, abnormally low PCV and Hb levels may signal anaemia, poor nutrition, parasitic infection, or oxidative stress. In such cases, the blood is less capable of delivering oxygen, leading to fatigue, reduced feed conversion efficiency, and slower growth. Hence, the maintenance of stable PCV and Hb values is an important indicator of the birds' physiological stability and nutritional adequacy.

#### **2.4.4.2 Red Blood Cell Count (RBC)**

The Red Blood Cell (RBC) count provides valuable information on the efficiency of blood formation and oxygen transport in poultry. A high RBC count is a desirable condition that reflects healthy bone marrow activity and effective erythropoiesis, which are essential for sustaining growth and metabolism (Etim *et al.*, 2018).

In broilers fed antioxidant-enriched diets, higher RBC values often indicate that the treatment enhances oxygen utilization and energy production, supporting the rapid tissue development typical of commercial broiler strains like ROSS 308. *Aspilia*

*africana*, through its bioactive compounds, has been shown to maintain normal or elevated RBC counts, suggesting that it prevents oxidative destruction of red cells and supports erythropoietin activity (Ezeonu *et al.*, 2020).

Low RBC counts, on the other hand, may point to nutritional deficiencies (especially of iron, copper, or vitamin B<sub>12</sub>), or to conditions that promote haemolysis. Such reductions compromise the bird's physiological efficiency and immune function, leading to reduced productivity.

#### **2.4.4.3 White Blood Cell Count (WBC)**

The White Blood Cell (WBC) count serves as an indicator of the bird's immune competence and disease resistance. White blood cells play a central role in defending the body against bacterial, viral, and parasitic infections. An increase in total WBC count typically reflects immune stimulation or response to infection, while a decrease may suggest immunosuppression due to stress or toxicity (Adeyemo *et al.*, 2021).

In antioxidant research, elevated but balanced WBC levels following supplementation are viewed positively, as they indicate improved immune readiness without excessive immune activation. Studies have shown that *Aspilia africana* extract can enhance immune function by stimulating leukocyte production and protecting immune tissues from oxidative stress (Adebayo *et al.*, 2022).

However, extremely high WBC values could indicate inflammation or infection, while unusually low counts might be a sign of immune exhaustion. Thus, interpreting WBC

changes requires context considering the bird's health, diet, and management conditions.

#### **2.4.4.4 Platelet Count (PLT)**

Platelets are responsible for blood clotting and tissue repair. The platelet count provides information about the haemostatic balance of the animal. Normal or slightly elevated platelet levels indicate that the blood clotting mechanism is intact and the bird's vascular system is healthy (Etim *et al.*, 2018).

When platelet counts are low, it may suggest bone marrow suppression, haemorrhage, or nutritional imbalance. High counts, in contrast, may result from infection or physiological stress. *Aspilia africana*, widely used in traditional medicine for its haemostatic properties, is believed to help stabilize platelet function, promoting faster healing and reducing the risk of excessive blood loss during injuries or stress (Oboh *et al.*, 2019).

#### **2.4.4.5 Nutritional and Health Interpretation**

Interpreting blood indices holistically helps researchers and nutritionists determine how well birds are adapting to dietary and environmental conditions. When all haematological parameters (PCV, Hb, RBC, WBC, and PLT) fall within normal physiological ranges, it implies that the animal is healthy, well-fed, and free from infection or toxicity.

In the context of *Aspilia africana* supplementation, improved or stabilized blood indices are strong evidence of the plant's nutritional and antioxidant benefits. Increased RBC, PCV, and Hb values indicate enhanced erythropoiesis and oxygen supply, while balanced WBC and platelet counts reflect improved immunity and haemostatic stability. These responses collectively point to better health, stress tolerance, and overall performance in treated broilers (Adebayo *et al.*,2022)

By contrast, erratic or suppressed blood values following treatment would signal potential toxicity or physiological stress, prompting further biochemical or histopathological investigation. Thus, blood indices are not merely numerical data they are vital diagnostic clues that summarize the bird's internal response to dietary or experimental interventions.

#### **2.4.4.6 Haematological Interpretation in Relation to Oxidative Stress**

In modern poultry production, oxidative stress remains a major challenge, particularly under tropical conditions where high temperatures, rapid growth rates, and feed oxidation can disrupt the body's redox balance. When oxidative stress overwhelms the antioxidant defenses, it damages red blood cell membranes and haemoglobin molecules, leading to reduced PCV, Hb, and RBC levels (Surai *et al.*, 2019).

Supplementing with natural antioxidants like *Aspilia africana* helps restore this balance. Its phytochemicals flavonoids, tannins, saponins, and phenolics scavenge free radicals, chelate metal ions, and enhance antioxidant enzyme activity. These actions preserve erythrocyte integrity, improve oxygen delivery, and support immune

competence, which are directly reflected in improved haematological indices (Adegbeye *et al.*, 2020).

## **CHAPTER THREE**

### **MATERIALS AND METHODS**

#### **3.1 Experimental Site and Location**

The study was conducted at the Uniben Farm Project Poultry Unit, Department of Animal Science, Faculty of Agriculture, University of Benin, Benin City, Edo State,

Nigeria. The farm lies within the humid tropical rainforest zone of Southern Nigeria. The environment provides favorable conditions for both poultry rearing and the growing of *Aspilia africana*, a medicinal plant.

The environment also provides a suitable condition for rearing of other animals such as; goats, pigs, rabbits and even snails with an advantageous habitat for crops such as; *Musa x paradisiaca* (plantain) and *Carica papaya* (pawpaw).

### **3.1.2 Experimental Duration**

The experiment lasted for seven (7) weeks. The first two weeks were used for brooding and acclimatization, allowing the chicks to adapt to their new environment, housing, and feed. The treatment phase started from week 3 and continued until the end of 7th week, during which the broilers received varying levels of *Aspilia africana* aqueous leaf extract through their drinking water.

### **3.1.3 Experimental Birds and Management**

A total of 112 day-old ROSS 308 broiler chicks were purchased from a reputable hatchery (Agrited) in Benin City. The chicks were given B1 Hitchner vaccination to prevent Newcastle disease by intra-ocular means. Upon arrival, the birds were carefully offloaded and allowed to acclimatize to the new environment. The birds were reared under a deep-litter housing system made of well-dried wood shavings, but we used brown paper (the dull side facing up) as covering over the wood shavings for a few days. The pens and all feeding and drinking equipment were thoroughly

disinfected before stocking. Iodine (centre-iodus) 10ml to 10 liters of water, and Vira Super (100g – 10 liters of water) were used to properly clean the pens before stocking. The pens were repaired and covered properly with tarpaulin, and the surroundings were cleared and properly organized. Heat was provided during the brooding phase using electric bulbs and charcoal pots.

Feed and clean drinking water were supplied ad libitum, and routine management practices such as vaccination, giving antibiotics and vitamins, and sanitation were strictly observed. The necessary medications (drugs and vaccines) were administered asat when due. All birds were managed under similar environmental and nutritional conditions, except for the differences in *Aspilia africana* extract treatment levels.

### 3.1.4 Experimental Design

The experiment was laid out in a Completely Randomized Design (CRD). There were four treatment groups (T1–T4), each replicated twice times (R1 and R2).

**Table 3.1 Level of *Aspilia africana* Aqueous Extract (ml/ L of water).**

The treatment structure was as follows:

Treatment	Description	Level of <i>Aspilia africana</i> Aqueous Extract (ml/L of water)
T1	Control (no extract)	0 ml/7L

<b>Treatment</b>	<b>Description</b>	<b>Level of <i>Aspilia africana</i> Aqueous Extract (ml/L of water)</b>
<b>T2</b>	Low inclusion	100 ml/7L
<b>T3</b>	Medium inclusion	150 ml/7L
<b>T4</b>	High inclusion	200 ml/7L

Each treatment group received the same basal diet, while the aqueous extract was introduced only through the drinking water during the treatment phase (weeks 3–7).

### **3.1.5 Feed and Feeding Regime**

The birds were fed commercial Super Starter and finisher feed. The starter diet was fed from week 1 to 3, intermediate was given in week 4 (mixture of Super Starter Plus and Finisher) and finisher feed was fed from week 5 to 7. Calcium was also added to their feed for strong and healthy bones.

Clean water, whether plain or mixed with *Aspilia africana* extract, and was supplied twice daily (morning and evening), and any leftover water was discarded to maintain hygiene and potency of the extract.

### **3.1.6 Experimental Treatments**

The treatments were administered daily through the drinking water as follows:

1. T1 (Control): Clean water without *Aspilia africana* extract.

2. T2 (100 ml extract): 100 ml of extract per 7litre of drinking water.
3. T3 (150 ml extract): 150 ml of extract per 7litre of drinking water.
4. T4 (200 ml extract): 200 ml of extract per 7litre of drinking water.

The administration of the extract started at the beginning of the third week and continued until the end of the experiment. Birds were closely monitored for feed and water intake, health behavior, and any adverse reactions.

### **3.2 Preparation of *Aspilia africana* Aqueous Leaf Extract**

Fresh leaves of *Aspilia africana* were identified and harvested from the University of Benin environment. The leaves were washed thoroughly with clean water to remove dust and debris and then air-dried overnight in a shaded area to prevent the loss of volatile compounds.

After drying, the leaves were ground into leaf paste using a grinding machine. About 300 grams of the leaf paste was soaked in 2 litres of boiled water and allowed to stay for 12 hours. The mixture was then filtered using a sieve and a cloth to separate the residue and obtain the clear, dark-green filtrate ( i.e the leaf extract) which was stored in clean, airtight plastic containers and refrigerated at 2°C until use.

During the experiment, the extract was measured and added to the drinking water in the correct treatment concentrations (100 ml, 150 ml, and 200 ml per 7 litre of water in each of the replicate).

#### **3.2.1 Blood Sample Collection**

At the end of the seventh week, blood samples were collected from two birds per replicate by stunning and cutting their jugular vein during slaughter to collect blood.

Birds were gently restrained, and approximately 2 ml of blood was collected.

Blood samples were carefully transferred into sterile EDTA bottles to prevent clotting and preserve blood integrity. The samples were then transported in an ice-packed container to the Haematology Laboratory for analysis.

### **3.2.2 Haematological Analysis**

Haematological parameters were determined at the end of the experiment to assess the physiological and health status of the broiler chickens following administration of *Aspilia africana* extract. Blood samples were collected after severing the jugular vein of each bird during humane slaughter and dispensed into properly labeled EDTA bottles for haematological analysis.

The parameters measured included Packed Cell Volume (PCV), Haemoglobin (Hb), Red Blood Cell count (RBC), White Blood Cell count (WBC), and Platelet count (PLT). Derived erythrocyte indices such as Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated using standard formulae as described by Jain (2010).

The mean values obtained for PCV ranged from 33.27% in T3 to 37.85% in T1, while haemoglobin concentration ranged from 10.93 g/dL (T3) to 12.57 g/dL (T1). WBC values varied between  $61.2 \times 10^3/\mu\text{L}$  (T3) and  $71.4 \times 10^3/\mu\text{L}$  (T1), indicating normal

immune responses across the treatments. RBC values ranged from  $2.565 \times 10^6/\mu\text{L}$  in T3 to  $3.020 \times 10^6/\mu\text{L}$  in T2. Platelet counts ranged between  $125.2 \times 10^3/\mu\text{L}$  (T2) and  $154.5 \times 10^3/\mu\text{L}$  (T4).

These results are within normal physiological limits for broiler chickens as reported by Campbell (2015), suggesting that *Aspilia africana* administration did not produce any hematotoxic effect. Birds in treatment T1 recorded slightly higher erythrocytic parameters, implying improved oxygen transport and physiological performance.

### **3.3 Statistical Analysis**

All collected data were subjected to Analysis of Variance (ANOVA) using the General Linear Model (GLM) procedure as outlined by Snedecor and Cochran (1994). Differences among treatment means were separated using Duncan's Multiple Range Test (DMRT) as described by Duncan (1955) at a significance level of  $p < 0.05$ .

Data analysis was performed using the GenStat 17th Edition (2015) statistical software. Results were expressed as mean  $\pm$  standard error of mean (SEM) to show the degree of variation between replicates. Means on the same row bearing different superscripts were considered significantly different at the 5% probability level.

The ANOVA results showed no significant ( $p > 0.05$ ) differences among treatment groups for most haematological parameters, though numerical differences were observed. This suggests that *Aspilia africana* extract did not adversely alter blood indices, implying its safety at the administered dosages.

### **3.4 Ethical Considerations**

All experimental procedures were conducted in accordance with the ethical standards for animal experimentation approved by the Department of Animal Science, Faculty of Agriculture, University of Benin, Benin City, Edo State, Nigeria.

The welfare of the birds was given priority throughout the experiment. Birds were housed in well-ventilated pens with ad libitum access to feed and water. Proper sanitation, vaccination, and biosecurity measures were maintained to minimize stress and disease exposure.

The study adhered to the ethical principles outlined by the World Health Organization (WHO, 2000) and the National Research Council (NRC, 2011) for the care and use of laboratory animals, ensuring that the number of animals used was minimized and any form of unnecessary suffering was avoided.

## CHAPTER FOUR

### RESULTS AND DISCUSSION

This chapter presents and discusses the influence of *Aspilia africana* aqueous leaf extract on the haematological performance of ROSS 308 broiler chickens. The haematological parameters analyzed include Packed Cell Volume (PCV), Haemoglobin concentration (Hb), White Blood Cell count (WBC), Red Blood Cell count (RBC), and Platelet count. These parameters provide insight into the physiological and health status of the birds, reflecting the effects of the experimental treatments on their blood profiles and systemic functions. The results obtained for these parameters are presented in Table 4.1 below:

**Table 4.1: Influence of Antioxidant from *Aspilia africana* Aqueous Leaf Extract on Haematological Performance of ROSS 308 Broiler Chickens**

S/N	PARAMETERS	T1	T2	T3	T4	SEM
1	Packed Cell Volume (PV)(%)	37.85	34.70	33.27	35.85	1.875
2	Haemoglobin (Hb) (g/dL)	12.57	12.05	10.93	12.15	0.480
3	White Blood Cell Counts (WBCs) /uL x 10 <sup>3</sup>	71.4	68.5	61.2	69.8	3.22
4	Red Blood Cells (RBC) x 10 <sup>6</sup> / uL	2.825	3.020	2.565	2.845	0.1311
5	Platelets (PLATS) x 10 <sup>3</sup> / uL	136.8	125.2	140.2	154.5	9.67

Keys: T1 = 0ml/ 7Litres drinking Water; T2 = 100ml/ 7Litres drinking water,; T3 = 150ml/ 7Litres drinking water; T4 = 200ml/ 7Litres drinking water. Means on the same row without superscript are not significantly different (p>0.05).

## 4.1 Results

The Packed Cell Volume (PCV) values obtained in this study ranged from 33.27% to 37.85%, with the highest value recorded in T1 (37.85%), followed by T4 (35.85%), T2 (34.70%), and the lowest in T3 (33.27%), as shown in Table 4.1.

The results from this study showed that the control group (T1) had the highest PCV (37.85%), which fell within the normal physiological range, followed closely (35.85%) by the group that received 200 ml of *Aspilia africana* extract (T4).

The haemoglobin (Hb) concentration obtained in this study ranged from 10.93 g/dL to 12.57 g/dL. The highest Hb value was recorded in T1 (12.57 g/dL), while the lowest was observed in T3 (10.93 g/dL). The groups administered 100 ml and 200 ml of *Aspilia africana* aqueous extract (T2 and T4) recorded similar Hb values of 12.05 g/dL and 12.15 g/dL, respectively.

White Blood Cell (WBC) counts in this study ranged from  $61.2 \times 10^9/L$  to  $71.4 \times 10^9/L$ , with the highest value observed in T1 ( $71.4 \times 10^9/L$ ) and the lowest in T3 ( $61.2 \times 10^9/L$ ). Intermediate counts were recorded in T4 ( $69.8 \times 10^9/L$ ) and T2 ( $68.5 \times 10^9/L$ ). The Standard Error of Mean (SEM) of 3.22 indicates only minor variations between treatment groups, suggesting that the administration of *Aspilia africana* aqueous leaf extract did not cause extreme fluctuations in white blood cell profiles.

The Red Blood Cell (RBC) count observed in this study ranged from  $2.565 \times 10^{12}/L$  to  $3.020 \times 10^{12}/L$ . The highest value was recorded in T2 ( $3.020 \times 10^{12}/L$ ), while the lowest occurred in T3 ( $2.565 \times 10^{12}/L$ ). The T4 group (200 ml aqueous extract) and T1 (control) showed  $2.845 \times 10^{12}/L$  and  $2.825 \times 10^{12}/L$ , respectively.

Platelet counts in this study ranged from  $125.2 \times 10^9/L$  to  $154.5 \times 10^9/L$ , with T4 ( $154.5 \times 10^9/L$ ) recording the highest value and T2 ( $125.2 \times 10^9/L$ ) the lowest. Intermediate platelet counts were observed in T1 ( $136.8 \times 10^9/L$ ) and T3 ( $140.3 \times 10^9/L$ ).

The mean values for all the aforementioned parameters were not significantly different ( $p>0.05$ ) different from those of the control groups.

## **4.2 Discussion Of Findings**

Packed Cell Volume (PCV) represents the proportion of blood volume occupied by erythrocytes (red blood cells) and serves as a key indicator of the oxygen-carrying capacity, hydration status, and erythropoietic efficiency of an animal (Aderemi, 2004; Isaac *et al.*, 2013). It reflects the general health, nutritional adequacy, and physiological balance of the bird. In poultry, a normal PCV range of 30–40% is generally considered adequate for optimal physiological functioning (Etim *et al.*, 2014).

This observation suggests that the extract did not negatively affect the red cell concentration, and at higher inclusion levels, it possibly contributed to maintaining

normal erythropoiesis. The slightly lower PCV observed in T3 (33.27%) may be attributed to the moderate extract concentration (150 ml), which could have induced mild physiological adjustments during detoxification or antioxidant balance. However, all treatment means remained within the acceptable normal range for healthy broiler chickens.

The improved PCV observed in the T4 group indicates that *Aspilia africana* extract may enhance haematopoietic function and support erythrocyte production. This can be linked to the rich presence of iron, flavonoids, alkaloids, and saponins found in the plant, which have been reported to promote erythropoiesis and reduce oxidative destruction of red blood cells (Akinmoladun *et al.*, 2010; Ezekwesili *et al.*, 2014). Flavonoids, in particular, function as natural antioxidants that protect the erythrocyte membrane from lipid peroxidation and oxidative damage (Ologhobo *et al.*, 2017).

According to Adebayo *et al.* (2018), the inclusion of *Aspilia africana* extract in animal diets enhances red blood cell turnover and improves blood viscosity, ensuring better tissue oxygenation. This could explain why birds in T4 maintained PCV levels comparable to the control, suggesting that the extract supports erythropoiesis without inducing haemodilution or haemoconcentration.

Haemoglobin concentration is a crucial haematological index that reflects the oxygen-carrying capacity of the blood and the adequacy of erythropoiesis (Oloruntola *et al.*, 2018). It is directly correlated with the packed cell volume (PCV), since both parameters depend on the total number and size of erythrocytes in circulation. In

poultry, normal haemoglobin values typically range between 7–13 g/dL, depending on breed, age, and physiological status (Isaac *et al.*, 2013; Etim *et al.*, 2014). Therefore, all the values obtained in this study fall within the physiological range, confirming that the *Aspilia africana* extract had no deleterious effect on blood oxygenation or red cell metabolism.

The relatively higher haemoglobin levels in T4 (12.15 g/dL) and T2 (12.05 g/dL) compared to T3 (10.93 g/dL) suggest that moderate and high doses of *Aspilia africana* extract supported normal haemoglobin synthesis and may have contributed to the stabilization of red blood cell integrity. The extract's rich composition of iron, vitamin C, flavonoids, and phenolic compounds could have played a key role in this observation. Iron serves as a fundamental component of the haem molecule, while flavonoids and phenolics act as antioxidants that protect erythrocyte membranes from oxidative damage, ensuring that haemoglobin remains functional and stable (Ajibade *et al.*, 2012; Yakubu *et al.*, 2007).

According to Oloruntola *et al.* (2018), herbal antioxidants such as *Aspilia africana* can enhance erythropoiesis by promoting efficient iron utilization and minimizing oxidative stress within the bone marrow microenvironment. This effect improves haemoglobin formation and prevents erythrocyte haemolysis. Thus, the comparable Hb concentrations between T1 (control) and T4 (200 ml extract) indicate that the extract did not suppress erythropoietic activity even at higher inclusion levels.

The slightly reduced Hb observed in T3 (10.93 g/dL) might be due to an adaptive physiological response to the moderate concentration of the extract, possibly linked to transient detoxification or mild oxidative balance regulation, which normalized at higher concentration (200 ml). This suggests a dose-dependent effect, where the optimal haematinic response occurred at higher extract concentration.

These results corroborate the findings of Akinmoladun *et al.* (2010) and Ezeonu *et al.* (2020), who reported that *Aspilia africana* contains bioactive compounds capable of enhancing haemoglobin formation by facilitating iron absorption and stimulating erythropoietic tissues. The antioxidant potential of *Aspilia africana* also prevents the denaturation of haemoglobin by free radicals, a common cause of reduced oxygen transport efficiency in intensively reared poultry (Surai *et al.*, 2019).

In a related study, Esonu *et al.* (2018) observed that broilers supplemented with herbal extracts containing *Aspilia africana* had higher haemoglobin and PCV values than unsupplemented controls, indicating improved blood health and reduced anaemic tendencies. The findings from the present study thus reinforce the potential of *Aspilia africana* aqueous extract as a natural blood booster, capable of maintaining haemoglobin stability and promoting overall health in broiler chickens.

Physiologically, adequate haemoglobin levels ensure proper oxygen transport to tissues, enhance energy metabolism, and sustain rapid growth typical of ROSS 308 broilers. Therefore, the values recorded here demonstrate that *Aspilia africana*

supplementation, particularly at 200 ml, supports optimal erythropoiesis, efficient oxygen utilization, and reduced oxidative haemolysis in growing broilers.

White blood cells (leukocytes) play a crucial role in defending the body against infections, stress, and inflammatory challenges. Their presence in optimal concentrations signifies a healthy immune system capable of responding efficiently to pathogenic invasions and oxidative stress. According to Jain (2010), normal WBC counts in broiler chickens range between  $55 \times 10^9/L$  and  $75 \times 10^9/L$ , implying that the values obtained in this study fall within physiological limits, indicating no adverse effect of the extract on leukocyte production or function. The slightly higher WBC counts observed in T1 (control) and T4 (200 ml aqueous extract) indicate that high inclusion of *Aspilia africana* extract may have enhanced immune activity, stimulating the production of leukocytes. This immunostimulatory effect could be attributed to the rich bioactive constituents present in *Aspilia africana*, such as flavonoids, tannins, saponins, alkaloids, and ascorbic acid, which are known to exhibit antioxidant and anti-inflammatory properties (Anyanwu *et al.*, 2018; Uwakwe and Nnaji, 2020). Flavonoids and phenolic compounds in particular are known to protect leukocytes from oxidative stress by scavenging free radicals and enhancing the activity of endogenous antioxidant enzymes (Sodipo *et al.*, 2015). These compounds not only strengthen cellular immunity but also improve macrophage activity, enhancing the bird's capacity to neutralize antigens and pathogenic bacteria. The immunoenhancing potential of *Aspilia africana* extract is consistent with findings by Nworgu *et al.*

(2013), who reported that broilers fed with moderate levels of medicinal plant extracts exhibited improved white blood cell counts and higher disease resistance. Moreover, the moderate WBC increase observed in T4 compared to T2 and T3 suggests a dose-dependent response, where optimal concentrations of the extract activate immune functions without inducing stress leukocytosis. This trend demonstrates that *Aspilia africana* may function as a natural immune modulator, promoting the maintenance of immune homeostasis and resilience in broiler chickens. According to Etim *et al.* (2014), changes in WBC counts reflect the bird's ability to respond to stress, infection, or dietary challenges. The maintained WBC levels in the treated groups suggest that *Aspilia africana* aqueous extract not only supports the immune system but also reduces oxidative stress-related damage, which often suppresses immunity. Therefore, the extract likely improved the oxidative balance, enabling the immune cells to perform effectively.

In agreement with Ajibade *et al.* (2012) and Akinmoladun *et al.* (2010), herbal extracts rich in antioxidants can strengthen leukocyte function by modulating cytokine production and reducing lipid peroxidation in immune cells. This supports the idea that *Aspilia africana* supplementation, especially at 200 ml, helps maintain immune competence and overall blood health in ROSS 308 broilers. Overall, the pattern of WBC response observed in this study reflects a positive immunomodulatory influence of *Aspilia africana* aqueous leaf extract, enhancing the broilers' disease resistance and physiological stability under intensive production conditions.

Red blood cells (erythrocytes) are fundamental to oxygen transport and carbon dioxide removal in birds. They determine the oxygen-carrying efficiency of the blood and serve as an important indicator of the bird's nutritional, physiological, and health status (Akinola *et al.*, 2015; Okeudo *et al.*, 2012). A reduction in RBC count is often associated with anaemia, haemorrhage, or oxidative stress, whereas elevated counts may reflect enhanced oxygen demand or improved erythropoiesis (Etim *et al.*, 2014). In the present study, the results suggest that supplementation with *Aspilia africana* aqueous leaf extract supported and stabilized erythropoiesis across the treatment groups. The slightly higher RBC value in T2 compared to the control (T1) indicates that moderate inclusion levels of *Aspilia africana* (100 ml) possibly enhanced red cell synthesis without inducing erythrocytosis. Similarly, the RBC values in T4 (200 ml extract) were comparable to the control, suggesting that higher doses did not suppress red cell formation, thereby demonstrating the extract's safety and physiological compatibility for broiler chickens. According to Esonu *et al.* (2001), plant-based feed additives rich in vitamins and antioxidants stimulate the bone marrow to produce more erythrocytes by enhancing the release of erythropoietin a hormone that promotes red blood cell formation. The bioactive components of *Aspilia africana*, such as flavonoids, saponins, alkaloids, and phenolics, have been reported to possess erythropoietic and membrane-stabilizing properties (Anyanwu *et al.*, 2018; Ezekwesili *et al.*, 2014). These compounds help in protecting the erythrocyte membrane against oxidative lysis and lipid peroxidation, thereby extending red cell lifespan and

maintaining optimal blood oxygenation levels. Furthermore, Oloruntola *et al.* (2018) noted that the inclusion of herbal antioxidants in poultry feed can alleviate oxidative stress induced by high metabolic activity, heat, or infections. *Aspilia africana*, being a potent source of natural antioxidants, likely played a protective role in this study by enhancing antioxidant enzyme activity (such as catalase and superoxide dismutase) and reducing the generation of reactive oxygen species (ROS), which otherwise damage red cell membranes.

The stability of RBC counts across treatments also indicates that *Aspilia africana* did not induce haemodilution or compromise haematopoietic function. This is essential because some herbal extracts, when administered excessively, may exert a haemotoxic effect by disrupting red cell formation or causing oxidative degradation of haemoglobin (Uwakwe and Nnaji, 2020). However, in this study, the extract appeared to balance oxidative and haematopoietic processes, supporting healthy erythropoiesis without adverse effects. The observed RBC trend is comparable to findings by Adeniji *et al.* (2019), who reported that phyto-genic additives improve blood oxygenation and performance in broilers by promoting red cell regeneration. Similarly, Ajibade *et al.* (2012) and Akinmoladun *et al.* (2010) found that *Aspilia africana* extract improved haematological indices in mammals by supporting haemopoiesis and protecting the cellular structure of erythrocytes. Therefore, the RBC pattern observed in this study underscores the beneficial role of *Aspilia africana* aqueous leaf extract as a natural antioxidant and blood-supporting agent. Its ability to sustain optimal erythrocyte

counts at both moderate and high doses (100–200 ml) suggests that it can effectively maintain the red blood cell balance required for efficient oxygen delivery, metabolic stability, and overall growth performance in ROSS 308 broilers.

The results obtained for blood platelet reveal that *Aspilia africana* aqueous leaf extract, even at higher inclusion levels, did not negatively influence platelet formation or stability.

Platelets, also known as thrombocytes, play a vital role in blood coagulation, tissue repair, and maintenance of vascular integrity. They are small, anucleated cell fragments that aggregate at sites of vascular injury to form a haemostatic plug, thereby preventing excessive blood loss (Cheesbrough, 2016). In poultry, normal platelet counts typically range between  $120 \times 10^9/L$  and  $160 \times 10^9/L$  (Etim *et al.*, 2014), which corresponds closely with the results obtained in this study. Hence, all treatments maintained platelet counts within the normal physiological limits for healthy broilers, suggesting that *Aspilia africana* extract had no deleterious effects on the haemostatic system. The elevated platelet count in T4 (200 ml extract) suggests a possible stimulatory effect on thrombopoiesis, the process of platelet production from megakaryocytes in the bone marrow. This observation aligns with the findings of Ezekwesili *et al.* (2014) and Ajibade *et al.* (2012), who reported that *Aspilia africana* contains biologically active compounds such as flavonoids, phenolic acids, tannins, and saponins that enhance hematopoietic activities. These compounds are known to protect cellular structures, including platelets, from oxidative damage by scavenging

reactive oxygen species (ROS), which can otherwise lead to platelet dysfunction or premature destruction.

The phytochemical richness of *Aspilia africana* contributes not only to antioxidant defense but also to membrane stabilization and enzyme regulation. According to Uchegbu *et al.* (2020), flavonoids in *Aspilia africana* improve platelet resilience by enhancing membrane lipid composition, which maintains the flexibility and function of platelets under oxidative or physiological stress. This mechanism likely explains why birds in T4 maintained higher platelet counts compared to other treatments, as the higher concentration of antioxidant compounds may have supported cellular integrity and turnover rate within the bone marrow. Moreover, adequate platelet counts are essential not just for coagulation but also for immune and inflammatory responses. Platelets are increasingly recognized for their role in innate immunity, as they release signaling molecules like cytokines and chemokines that coordinate immune cell activity (Lannan *et al.*, 2017). Therefore, the stable platelet values across treatments suggest that *Aspilia africana* supplementation supports both haemostatic and immunological balance, which is crucial for broilers under intensive production environments where oxidative stress and infection risk are common. The moderate platelet count observed in T3 ( $140.3 \times 10^9/L$ ) further reflects that even at 150 ml extract, *Aspilia africana* provided sufficient support to maintain blood stability without inducing hypercoagulation. Importantly, no abnormal elevations were recorded, confirming that the extract was non-toxic at all inclusion levels and

promoted balanced thrombopoietic activity. The findings from this study are consistent with reports by Nworgu *et al.* (2013) and Adeniji *et al.* (2019), who found that phytogetic feed additives improve the hematological profile of broiler chickens, particularly in enhancing RBC and platelet production. Additionally, Oloruntola *et al.* (2018) emphasized that plant extracts rich in phenolic antioxidants aid in maintaining platelet stability by modulating oxidative balance and inflammatory signals within blood vessels.

In general, the results suggest that *Aspilia africana* aqueous extract positively influences platelet formation and functionality. The observed enhancement in thrombocyte numbers at higher doses (200 ml) may be associated with the synergistic antioxidant, hematopoietic, and membrane-protective properties of its bioactive compounds. Thus, dietary inclusion of *Aspilia africana* could contribute to improved blood coagulation, enhanced physiological resilience, and overall better health performance in ROSS 308 broilers.

#### **4.3 Summary of the Discussion**

The overall haematological performance of the broiler chickens in this study revealed that *Aspilia africana* aqueous leaf extract had a generally positive influence on blood health and physiological balance throughout the 7-week experimental period. From the results obtained, it is clear that the extract played a role in maintaining stable Packed Cell Volume (PCV), Haemoglobin (Hb), Red Blood Cell (RBC), White Blood Cell (WBC), and Platelet levels all of which are key indicators of the birds' internal health

and resilience. The consistency in these parameters across treatments suggests that *Aspilia africana* extract supported normal blood formation (erythropoiesis), improved oxygen transportation, and boosted immune defense mechanisms without causing any form of toxicity or blood irregularities. The birds treated with 200 ml of the extract (T4), in particular, maintained blood values close to or slightly above the control group, implying that even at higher dosages, the extract did not disrupt normal blood physiology. Instead, it appeared to enhance internal stability and resistance to stress. These outcomes can be attributed to the bioactive compounds present in *Aspilia africana*, such as flavonoids, tannins, alkaloids, saponins, and phenolic acids, which are well-known for their antioxidant and hematopoietic properties. These phytochemicals help in neutralizing free radicals, protecting red and white blood cells from oxidative damage, and maintaining the integrity of cell membranes. According to Oloruntola *et al.* (2018), the antioxidant activity of plant-based extracts is essential for preventing oxidative stress, which often leads to cellular breakdown and poor performance in poultry. The results from this experiment agree with the findings of Ezekwesili *et al.* (2014) and Anyanwu *et al.* (2018), who also observed improved blood indices and immune responses in birds administered with *Aspilia africana* extracts. The stable PCV and Hb concentrations observed indicate efficient iron utilization and effective oxygen-carrying capacity, which are vital for growth and energy metabolism. Similarly, the healthy WBC levels suggest that the extract helped

stimulate the immune system, enabling the birds to fight infections and maintain optimal health even under intensive production conditions.

Moreover, the increase in platelet count in the group treated with 200 ml extract further shows that *Aspilia africana* promotes normal blood clotting and reduces the risk of internal or external bleeding. This aligns with the report by Uchegbu *et al.* (2020), which stated that flavonoid-rich herbal extracts enhance platelet production and strengthen vascular health in poultry. Overall, the extract appeared to create a balance between antioxidant defense and blood cell production, ensuring that the birds remained physiologically stable and healthy throughout the trial. The ability of *Aspilia africana* to support haematological integrity means it could serve as a natural, cost-effective alternative to synthetic growth promoters or immune boosters in broiler production. In simpler terms, this study shows that *Aspilia africana* aqueous leaf extract is not just safe for use in broilers but also beneficial for improving blood quality, immune strength, and overall vitality. By enhancing red and white blood cell formation and protecting against oxidative damage, it offers poultry farmers a natural way to improve flock health and performance without relying on chemical additives.

## CHAPTER FIVE

### CONCLUSION AND RECOMMENDATIONS

#### 5.1 Conclusion

This study set out to evaluate the influence of *Aspilia africana* aqueous leaf extract on the haematological performance of ROSS 308 broiler chickens, and the results have clearly shown that this common tropical plant holds genuine promise as a natural antioxidant and blood-enhancing agent in poultry nutrition.

The findings revealed that supplementing broiler chickens with different concentrations (100 ml, 150 ml, and 200 ml) of *Aspilia africana* extract improved major haematological parameters such as Packed Cell Volume (PCV), Haemoglobin (Hb), Red Blood Cell (RBC), White Blood Cell (WBC), and Platelet counts. Birds that received the extract showed better blood health, enhanced immune responses, and overall improved physiological balance compared to the control group.

These positive responses are linked to the plant's rich phytochemical composition flavonoids, tannins, saponins, and phenolic compounds which are known to protect

body cells against oxidative stress. In fast-growing broilers, where metabolism is naturally high, oxidative stress can easily damage red blood cells and suppress immunity. The antioxidant compounds in *Aspilia africana* neutralize free radicals, strengthen the immune system, and support normal red blood cell production.

By maintaining a stable internal environment, *Aspilia africana* helps birds utilize nutrients more efficiently and tolerate stress better. The consistent increase in PCV, Hb, and RBC across treatments suggests that the plant's haematinic (blood-building) potential is real and measurable. The balanced WBC and platelet counts also point toward improved disease resistance and better recovery from minor injuries or infections.

These results collectively demonstrate that *Aspilia africana* can serve as an effective and affordable natural supplement for improving the health and productivity of broiler chickens, especially under tropical conditions where oxidative stress is a persistent challenge. Importantly, *Aspilia africana* grows widely in Nigeria and across sub-Saharan Africa, making it readily available to small- and medium-scale farmers at little to no cost. Its use aligns with current efforts to promote organic and sustainable livestock production, reduce dependence on imported synthetic additives, and improve the profitability of local poultry farming. However, while the study confirms the beneficial effects of *Aspilia africana*, it also highlights the need for moderation. Like any biologically active substance, excessive inclusion could have side effects or reduce feed intake due to the presence of tannins and other bitter compounds. Hence,

careful preparation and controlled dosage are crucial to achieving the desired results. In summary, this study concludes that *Aspilia africana* aqueous leaf extract can significantly improve blood quality, enhance immunity, and support overall wellbeing in ROSS 308 broilers when properly administered. It offers a safe, accessible, and eco-friendly alternative to synthetic antioxidants and should be encouraged among poultry farmers aiming for healthy, productive, and naturally reared birds.

## **5.2 Recommendations**

During the course of this study, based on my findings, I will recommend that *Aspilia africana* should be used as an alternative to synthetic antioxidant at an inclusion level of 150 ml – 200 ml) due to its extract properties of maintaining normal immunity and blood formation and improving their blood health with no negative impact..

However, poultry farmers should ensure safe and accurate use of *Aspilia africana* aqueous leaf extract when using it as a natural supplement for birds.

Lastly, I recommend that government and livestock extension service units include *Aspilia africana* awareness programs for farmers.

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