

Archives of Immunology Research and Therapy

Research Article

Evaluation of *Bauhinia forficata*, *Caulophyllum thalictroides*, and *Combretum micranthum* Leaf Powders as Alternatives to Antibiotic Growth Promoters in Broiler Diets

Kumar, Amsar¹, Alagbe, John Olujimi^{1,2*}, Babatunde Tanimomo³, Rufus, Adebisi Oluwafemi⁴

¹Department of Animal Nutrition and Biochemistry, Sumitra Research Institute, Gujarat, India.

²Department of Animal Science, Centre for Distance Learning and Continuing Education, University of Abuja, Gwagwalada, Nigeria

³Department of Animal Production and Health, Faculty of Veterinary Medicine, University of Abuja, Nigeria

⁴Department of Animal Science, Faculty of Agriculture, University of Abuja, Nigeria

***Corresponding Author:** Alagbe, John Olujimi, Department of Animal Science, Centre for Distance Learning and Continuing Education, University of Abuja, Gwagwalada, Nigeria

Received Date: 16 June 2026; **Accepted Date:** 06 July 2026; **Published Date:** 10 July 2026

Copyright: © 2026 Alagbe, John Olujimi, this is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

The global poultry industry's shift away from synthetic sub-therapeutic antibiotics has intensified the search for safe, biosecure, and effective phytochemical feed additives (PFAs). This study was conducted to evaluate the effects of three distinct medicinal plant leaf powders—*Bauhinia forficata*, *Caulophyllum thalictroides*, and *Combretum micranthum*—on the growth performance, feed efficiency, hematological traits, serum biochemistry, and carcass characteristics of Hubbard broiler chickens. A total of 500 day-old unsexed Hubbard chicks were randomly assigned to 5 dietary treatments in a Completely Randomized Design (CRD). Each treatment consisted of 100 birds partitioned into 5 replicates (n=20 birds per replicate). The birds were reared in an environmentally controlled housing facility at the Gandhi College of Agriculture, Rajasthan, India. Dietary treatments comprised: T1 (Negative Control; basal diet only), T2 (Positive Control; basal diet + 10 g neomycin/kg), T3 (basal diet + 10 g *B. forficata* leaf powder/kg), T4 (basal diet + 10 g *C. thalictroides* leaf powder/kg), and T5 (basal diet + 10 g *C. micranthum* leaf powder/kg). Quantitative phytochemical profiling of the leaf powders was conducted via UV-Vis spectrophotometry, and the basal diet macro-nutrients were verified using a near-infrared (NIR) analyzer. Data were subjected to a one-way ANOVA, and means were separated using Duncan's Multiple Range Test ($p < 0.05$). Quantitative analysis revealed that *B. forficata* possessed the highest concentrations of flavonoids (209.1 mg/g) and phenols (186.0 mg/g). Broilers fed diets supplemented with botanical powders (T3–T5) exhibited significantly higher ($p < 0.05$) body weight gain and feed intake compared to the T1 and T2 groups. The Feed Conversion Ratio (FCR) was optimized and lowest in T3–T5, intermediate in T2, and highest in T1 ($p < 0.05$). Hematological variables and serum biochemical parameters (including total protein and globulin) were higher in the phytochemical groups (T3–T5) than in controls, except creatinine, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase concentrations which were not affected ($p > 0.05$). However, all values remained strictly within safe, non-toxic physiologic reference ranges. Carcass yield parameters—including dressed weight, dressing percentage, and the absolute weights of commercial cuts (breast, thighs, drumsticks)—were higher in T3–T5, intermediate in T2, and lowest in T1 ($p < 0.05$). In conclusion, dietary supplementation of *B. forficata*, *C. thalictroides*, and *C. micranthum* leaf powders at 10 g/kg safely enhances performance and tissue accretion in Hubbard broilers, completely outperforming conventional neomycin antibiotic growth promoters. *Bauhinia forficata* represents the most potent candidate due to its rich antioxidant phenolic compounds, making it a viable, high-yield phytochemical feed additive for commercial broiler formulations.

Keywords: Phytochemicals, *Bauhinia forficata*, Broiler performance, Carcass yield, Hematology, Antibiotic alternatives.

Introduction

The global poultry industry has undergone rapid intensification over the last several decades, evolving into one of the most efficient suppliers of high-quality animal protein worldwide [1]. To sustain this intensive production rate and meet the soaring global demand for animal protein, commercial broiler production heavily leverages fast-growing strains like the Hubbard broiler [2]. These modern strains are characterized by rapid muscle accretion, high volumetric feed consumption, and highly specialized metabolic demands [3]. However, this intensive genetic selection renders broilers highly vulnerable to gastrointestinal disorders, environmental stressors, and subclinical pathogen challenges [3]. Historically, these vulnerabilities were managed through the routine, sub-therapeutic dietary inclusion of Antibiotic Growth Promoters (AGPs), such as neomycin. AGPs function by non-selectively suppressing the gut microflora, reducing the thickness of the intestinal mucosal layer, and minimizing localized inflammatory responses, thereby shifting metabolic resources toward growth and muscle deposition [4]. Despite their proven economic efficacy, the unchecked use of sub-therapeutic antibiotics in livestock production has generated severe global public health concerns [5]. The primary issue is the rapid development of antimicrobial resistance (AMR) in both animal and human pathogens, alongside the risk of chemical residues persisting in poultry meat [6]. This clinical hazard has led to sweeping legislative bans or strict restrictions on the use of AGPs in animal feed across the European Union, parts of Asia, and many developing nations [7]. Consequently, the commercial poultry sector faces the critical challenge of identifying safe, biosecure, and sustainable alternatives capable of maintaining or improving broiler growth performance, feed conversion efficiency, and carcass yield without compromising avian welfare or public safety. Among the various alternatives under evaluation, Phytogenic Feed Additives (PFAs)—commonly known as botanicals or phytobiotics—have emerged as highly promising candidates [8].

Phytogenics are plant-derived products containing secondary metabolites that exert various beneficial physiological and pharmacological effects when incorporated into animal feed [9]. The efficacy of PFAs is directly tied to their diverse chemical profiles, which include flavonoids, phenols, tannins, alkaloids, steroids, and saponins [9]. These bioactive compounds act synergistically within the avian gastrointestinal tract. For instance, plant phenols and flavonoids are potent antioxidants that scavenge reactive oxygen species (ROS) and reduce intestinal oxidative stress, maintaining epithelial tight junctions [10, 11]. Concurrently, saponins and alkaloids exert selective antimicrobial actions by altering the cell membrane permeability of opportunistic pathogens like *Escherichia coli* and *Salmonella spp.*, while leaving beneficial lactic acid bacteria largely uncompromised [11]. Further-

more, these secondary metabolites are known to stimulate the secretion of endogenous digestive enzymes—such as amylase, trypsin, and lipase—and structurally optimize gut morphology by increasing villus height and expanding the functional nutrient absorptive surface area [12]. Among the wide array of tropical and subtropical flora, *Bauhinia forficata*, *Caulophyllum thalictroides*, and *Combretum micranthum* present compelling profiles for evaluation in poultry nutrition [12]. *Bauhinia forficata* is recognized for its high phenolic and flavonoid content, which contributes to its strong antioxidant properties. *Caulophyllum thalictroides* is rich in alkaloids and saponins, which are associated with strong antimicrobial activities and immunomodulatory traits. *Combretum micranthum* features a balanced distribution of tannins and steroids that can modulate nutrient assimilation and metabolic pathways. While these medicinal plants have been investigated in traditional medicine, their comparative evaluation as whole-leaf powder additives alongside conventional antibiotics in intensive poultry production systems remains limited [13]. Prior evaluations of phytogenic feed additives containing similar biochemical profiles suggest that botanical secondary metabolites can effectively replicate or exceed the performance benefits of conventional antibiotics [13]. Previous research on related species within the *Bauhinia* genus has demonstrated that its dense concentration of polyphenols and flavonoids significantly reduces lipid peroxidation in the intestinal mucosa of monogastric animals, leading to an increase in villus height and a corresponding reduction in the Feed Conversion Ratio (FCR) [14]. Similarly, studies involving alkaloid-rich extracts from *Caulophyllum* species have documented localized antimicrobial effects capable of selectively inhibiting opportunistic enteric pathogens like *Escherichia coli* and *Clostridium perfringens*, mimicking the sanitizing mechanisms of traditional antibiotics without destroying beneficial lactic acid microflora [11]. Furthermore, evaluations of *Combretum* leaf extracts in poultry models have reported positive modulations in hematological indices, notably increasing total protein and globulin concentrations, which reflect an activated immune system and improved hepatic nitrogen metabolism. However, despite these separate findings, there remains a critical gap in literature regarding a direct, head-to-head comparison of whole-leaf powders from *Bauhinia forficata*, *Caulophyllum thalictroides*, and *Combretum micranthum* against a standardized antibiotic control like neomycin within a single, highly controlled production model [15].

This study is justified by the urgent need to identify accessible, biosecure, and sustainable alternatives to synthetic antibiotics that can maintain high zootechnical performance and carcass yields in the poultry sector. Ultimately, this research provides the poultry industry with verified data to support the transition toward antibiotic-free production, turning

indigenous botanical resources into functional components for modern poultry diets.

Materials and Methods

Experimental Site

The feeding trial was conducted at the Poultry Research Unit of the Department of Animal Nutrition and Biochemistry, Gandhi College of Agriculture, Bharatpur (321001), Rajasthan, India. The experimental site lies within a semi-arid climatic zone characterized by distinct seasonal variations. During the trial period, the average ambient room temperature was maintained at $26 \pm 2^\circ\text{C}$ following the initial brooding phase, with a relative humidity fluctuating between 45 % and 65 %. The macroclimate of the region during this period recorded an average diurnal ambient temperature ranging from 22°C to 34°C , with minimal regional rainfall, typical of the sub-humid to semi-arid agro-ecological plains of Eastern Rajasthan.

Ethical Approval

All experimental procedures, animal handling protocols, and surgical methods for carcass evaluation were reviewed, checked, and strictly authorized by the Institutional Animal Ethics Committee (IAEC) of Gandhi College of Agriculture, Rajasthan, India, under the formal ethical approval voucher number GCA/IAEC/2026/POULT-042. The trial was carried out in strict accordance with the guidelines outlined by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India, and ensuring maximum adherence to international standard animal welfare norms.

Collection, Identification, and Processing of Medicinal Plants

Fresh leaves of *Bauhinia forficata*, *Caulophyllum thalictroides*, and *Combretum micranthum* were collected from verified botanical resource plots and surrounding regional floras. The plant samples were individualistically clean-sorted and transferred to the Taxonomy and Herbarium Section of the institution for authentication and botanical identification. Voucher specimens were officially assigned under reference repository codes GCA-BF-091 (*B. forficata*), GCA-CT-092 (*C. thalictroides*), and GCA-CM-093 (*C. micranthum*). The collected leaves were washed with tap water followed by rinsed distilled water to eliminate physical contaminants, and then spread on slatted trays to air-dry uniformly under shade at room temperature (30°C) for 14 days until a constant moisture weight was attained. The completely crispy-dried leaves were subsequently pulverized into a fine meal using a commercial multi-functional stainless steel herbal hammer mill. The resulting leaf powders were passed through a 1.0 mm mesh screen sieve to achieve a standardized particle size distribution and stored in airtight, opaque polyethylene zip-lock bags at 4°C to prevent photo-oxidation of the secondary

metabolites prior to inclusion in the experimental diets.

Quantitative Phytochemical Analysis

The quantitative evaluation of bioactive secondary metabolites within the prepared leaf powders was conducted utilizing advanced spectrophotometric and chromatographic methodologies. Total flavonoids, phenols, tannins, alkaloids, steroids, and saponins were analyzed using Thermo Scientific GENESYS 150 UV-Visible Spectrophotometer (USA) operating across a spectral wavelength range of 190 to 1100 nm, featuring a spectral bandwidth of 2.0 nm, wavelength accuracy of ± 0.5 nm, and equipped with automated high-precision quartz micro-cuvettes (10 mm light path). Total phenolic compounds were calibrated against gallic acid standards using the Folin-Ciocalteu method at 765 nm. Total flavonoids were quantified using the aluminum chloride colorimetric method at 415 nm against quercetin equivalents. Saponins and tannins were mapped via specialized extraction kits and read at 544 nm and 725 nm respectively. Values were expressed uniformly in milligrams per gram (mg/g) of dry matter.

Proximate Analysis of Basal Diet

Proximate analysis of basal diet was carried out using Nobb Rapid Near Infra-Red Feed Analyzer (Model ASD 3006C, Netherlands). The kit was operated at a spectral range: 350 to 2500 nm, optical Resolution of ≤ 3 nm at 700 nm, ≤ 10 nm at 1400 to 2100 nm.

Pre-Experimental Operations

Two weeks before the scheduled placement of day-old chicks, the poultry house, battery cages, open-sided ventilation mesh, watering pipelines, and feeding troughs were subjected to rigorous physical cleaning and high-pressure washing. The entire open interior was disinfected using a commercial broad-spectrum virucidal and bactericidal compound (Morigad plus Aquaclean, ratio 2:1). The house was subsequently sealed and subjected to chemical fumigation by compounding potassium permanganate crystals (KMnO_4) with a 40% formalin solution at a standard double-strength ratio (40 g KMnO_4 :80 mL formalin per m^3). The facility was kept sealed for 72 hours and then opened for a 4 day thorough ventilation flush prior to chick arrival. The heating systems were activated 24 hours before placement to achieve a uniform ambient brooding baseline floor temperature of 32°C .

Animals and Their Management

A total of 500 day-old unsexed commercial broiler chicks of the Hubbard strain were purchased from a reputable local hatchery certified by the Indian Society of Animal Production. Upon immediate arrival at the facility, individual chick weights were taken using a high-precision, sensitive electronic analytical platform scale (Mettler Toledo, Model ICS425, Swit-

zerland, precision tolerance of $\pm 0.01\text{g}$). The chicks were systematically distributed into their pre-assigned pens. The birds were reared under standard management setups within a clean multi-tier battery cage housing configuration. The brooding temperature schedule was maintained at 32°C during the first week, and decreased by 2°C weekly until it stabilized at the standard room environment temperature of 26°C at the end of week 3. Continuous illumination (24 hours light cycle) was provided during the initial brooding phase, transitioning to a 23 hours light and 1 hour dark cycle for the remainder of the 28-day trial. Clean, unmedicated fresh drinking water and the formulated experimental diets were served ad libitum throughout the entire experimental production life span. All mandatory veterinary vaccination schedules against Newcastle Disease (Lasota) and Infectious Bursal Disease (Gumboro) were strictly carried out.

Experimental Design and Dietary Treatments

The 500 chicks were arranged in a Completely Randomized Design (CRD). The birds were randomly allotted into 5 dietary treatment groups (T1 to T5). Each treatment comprised 100 birds, further split into 5 replicates containing 20 birds per replicate cell cage. The basal experimental diets were formulated to meet or exceed the nutritional requirements for broilers as recommended by the National Research Council [17]. The structural layout of the five experimental groups was assigned as follows:

Treatment 1 (T1): Negative Control; received basal diet only (no additives).

Treatment 2 (T2): Positive Control; received basal diet supplemented with 10 g neomycin per kg of diet.

Treatment 3 (T3): Received basal diet supplemented with *Bauhinia forficata* leaf powder at 10 g/kg of diet.

Treatment 4 (T4): Received basal diet supplemented with *Caulophyllum thalictroides* leaf powder at 10 g/kg of diet.

Treatment 5 (T5): Received basal diet supplemented with *Combretum micranthum* leaf powder at 10 g/kg of diet.

Growth Performance Parameters

The feeding trial lasted for a total duration of 28 days. Feed Intake (FI): Calculated as the difference between the total quantity of feed offered and the weight of the feed refused at the end of the corresponding week:

$\text{FI (g)} = \text{Feed Offered} - \text{Feed Refused}$

Body Weight Gain (BWG): Calculated as the difference between the final body weight and the initial body weight for each specific feeding period:

$\text{BWG (g)} = \text{Body Weight final} - \text{Body Weight initial}$

Feed Conversion Ratio (FCR): Calculated on a replicate-pen basis as the mass of feed consumed divided by the total body weight gain achieved by the surviving birds within that specific pen over the 28-day duration. Mortality was monitored and documented on a daily basis.

Blood Analysis

On day 28 of the experimental trial, a total of 50 birds (10 birds per treatment group; 2 birds randomly selected per replicate pen) were chosen for hematological and serum biochemical profiling. Blood samples (3.0 mL per bird) were collected via the bronchial wing vein (vena cutanea ulnaris) using sterile hypodermic needles and syringes. For each selected bird, the collected blood was split equally into two distinct sample tubes:

Hematological Profiling: The first portion (1.5 mL) was transferred immediately into vacuum tubes containing Ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. These samples were processed within 2 hours of collection to determine Packed Cell Volume (PCV), Red Blood Cell (RBC) counts, Hemoglobin (Hb) concentration, and White Blood Cell (WBC) counts using an automated veterinary hematology analyzer (Mindray BC-2800VET, China).

Serum Biochemical Profiling: The second portion (1.5 mL) was collected in plain, non-additive vacuum tubes and allowed to clot at room temperature (26°C) for 4 hours. The tubes were then centrifuged at 3,000 rpm for 15 minutes at 4°C to separate clean serum. The harvested serum was pipetted into sterile Eppendorf tubes and stored at -20°C until analysis. Total serum protein and globulin concentrations were quantified using commercial diagnostic kits (Erba Diagnostics, Germany) on an automated clinical biochemistry analyzer (Erba XL-180, Germany).

Carcass Characteristics

Following the collection of blood on day 28, the same 10 randomly selected birds per treatment group ($n=50$ total birds) were subjected to standard carcass and cut-up part evaluation. The selected birds were fasted for 12 hours prior to slaughter, while clean drinking water remained available ad libitum. The pre-slaughter live weight of each bird was recorded and birds were humanely slaughtered by severing the jugular vein and carotid arteries, allowed to bleed completely for 3 minutes, and subsequently scalded in a water bath at 60°C for 45 seconds. De-feathering was performed manually, followed by the removal of the shanks and head. Evisceration was carefully performed by making a midline incision around the vent to extract all internal viscera and abdominal fat. The resulting dressed carcass was weighed to determine the absolute dressed weight. The dressing percentage was calculated relative to the pre-slaughter live weight using the following formula:

$\text{Dressing Percentage (\%)} = \frac{\text{Eviscerated Weight (g)}}{\text{Live Weight (g)}} \times 100\text{s}$

The eviscerated carcass was then partitioned into its commercial cut-up parts using standard poultry butchering procedures. The primary primal cuts—including the breast, thighs, drumsticks, and back—alongside non-carcass components (head and shanks) were individually separated and weighed using a high-precision digital scale.

Statistical Analysis

All generated raw datasets for growth performance (feed intake, body weight gain, and calculated FCR values), hematological variables, serum biochemistry traits, and carcass measurements were organized in spreadsheets and subjected to a one-way Analysis of Variance (ANOVA) for a Completely Randomized Design using Duncan Multiple Range Test of SPSS Statistical Software Suite (Version 26.0, IBM Corp., USA).

The underlying mathematical model employed was:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where: Y_{ij} represents the individual observation within the treatment, μ is the overall experimental population mean.

T_i is the fixed effect of the dietary leaf powder treatment ($i=1, 2, 3, 4, 5$).

ε_{ij} is the random residual experimental error term.

Results and Discussion

The quantitative phytochemical analysis of medicinal plants in Table 2 reveals the presence of several phyto-compounds or secondary metabolites across all three experimental leaf powders, with *Bauhinia forficata* showing the most prominent profile. *Bauhinia forficata* leaf powder demonstrated exceptionally high concentrations of flavonoids (209.1 mg/g) and phenols (186.0 mg/g), which are recognized for their intense antioxidant capacities [19]. These compounds act as direct free radical scavengers, shielding the fragile intestinal epithelial cells from oxidative damage and enhancing the cellular integrity of the gut lining [20,21]. While *Caulophyllum thalictroides* exhibited slightly lower phenolic content, it contained the highest concentration of alkaloids (30.3 mg/g), which can exert potent selective antimicrobial actions against opportunistic gut pathogens in the intestinal flora [22]. *Combretum micranthum* exhibited a more modest, yet balanced, distribution of phytochemicals. The presence of saponins and tannins across all three groups at moderate levels plays a vital role in modifying gut physiology [23]. Saponins increase the permeability of intestinal mucosal cells, which selectively facilitates nutrient uptake, while simultaneously acting as natural surfactants that inhibit protozoal growth [23]. Meanwhile, the modest tannin levels present (54.2 - 72.24 mg/g) are low enough to avoid anti-nutritional effects, instead forming transient complexes with dietary proteins that shield them from premature ruminal-like bacterial degradation in the upper gastrointestinal tract, ultimately maximizing downstream enzymatic assimilation [24].

The effect of plant extract supplementation on the growth performance of Hubbard broilers (0 – 28 d) (Table 2). Average daily weight gain and average daily feed intake values were highest in T3 – T5, intermediate in T2 and lowest in T1 ($p<0.05$). Conversely, feed conversion ratio was more in T1, intermediate in T2 and lower in T1 ($p<0.05$). The significant increase in body weight gain and feed intake observed in the

phytogenic groups (T3, T4, and T5) relative to the negative control (T1) and antibiotic control (T2) can be directly associated to enhanced diet palatability and gastrointestinal development [25]. The presence of flavonoids and phenolic compounds in *B. forficata*, *C. thalictroides*, and *C. micranthum* act as natural sensory appetizers, stimulating olfactory and gustatory receptors in the birds to drive higher feed consumption [26]. Beyond mere intake, the bioactive constituents of these powders stimulate the pro-enzymes secreted by the pancreas and small intestine. This increase in endogenous enzyme activity (such as amylase, trypsin, and lipase) accelerates the breakdown of complex carbohydrates, proteins, and lipids into highly absorbable fractions. This outcome is in agreement with the report of [25] who recorded a higher body weight gain and feed intake in broilers fed diet supplemented with papaya seed oil. [26] discovered an increase in intestinal villus height and a optimization of the crypt depth of broilers fed diet supplemented with *Polyalthia longifolia* leaf meal. Feed conversion ratio noted in groups T3 through T5. While neomycin (T2) successfully lowered feed conversion ratio compared to the basal diet (T1) by non-selectively suppressing the gut microbial load a classic antibiotic growth promoter mechanism it lacks the ability to actively improve gut morphology or tissue accretion. The natural feed additives outpaced the conventional antibiotic by providing both a sanitizing antimicrobial effect and a direct physiological boost to nutrient digestion and metabolic efficiency. The result obtained is in consonance with the report of [27].

The effect of plant extract supplementation on the carcass characteristics of Hubbard broilers (0 – 28 d) is revealed in Table 4. This study noted that dressed weight, dressing percentage, and the absolute/relative weights of major primal cuts (breast, thighs, drumsticks, back) as well as non-carcass parts (shank, head) were significantly higher in T3–T5, intermediate in T2, and lowest in T1. The superior dressing percentage in the plant-fed groups suggests a higher meat-to-bone/viscera ratio, maximizing clean yield for processors. Similarly, the significant increase in breast and thigh weights the most commercially valuable parts of the carcass is a logical downstream effect of the improved feed conversion ratio levels. Because the phytogenics optimized amino acid digestibility and absorption, more protein was channeled toward skeletal muscle accretion rather than being wasted as nitrogenous waste. The corresponding increases in the weights of the back, head, and shanks suggests an improved mineral absorption, particularly due to the presence of bioactive compounds which can mimic anabolic pathways to support bone formation mineralization. This uniform enhancement in both primary carcass yields and individual cut-up parts demonstrates that these phytogenic additives successfully optimize the biological conversion of feed into high-value tissue, making them highly effective alternatives to traditional antibiotic growth promoters in T2. The result obtained is in agreement with the report of [28] when mango

leaf powder was supplemented in the diet of broilers. Similar outcome was recorded by [29] when phytogetic feed additive was included in the diet of broiler chickens.

Pack cell volume, red blood cell, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentrations, white blood cell, lymphocytes and monocytes were higher ($p < 0.05$) for T3-T5 than for T2 and T1 (Table 5). Blood parameters are highly sensitive indicators of the nutritional, immunological, and health status of farm animals. The higher values observed for haematological (e.g., pack cell volume, red blood cell, haemoglobin and white blood cell) in T3–T5 relative to T1 and T2 underscore the systemic benefits of the leaf powders. However, all haematological values for T3–T5 remained strictly within the established normal reference ranges for healthy broiler chickens [30]. This proves that at an inclusion rate of 10 g/kg diet, these leaf powders exert no hepatotoxic, nephrotoxic, or systemic physiological stress on the birds, confirming their safety as feed ingredients. Enhanced red blood cell counts and haemoglobin concentration imply increase erythropoiesis and oxygen-carrying capacity, supporting the intense metabolic demands of fast-growing Hubbard broilers [31]. The higher white blood cell profiles reflect an active, primed immune system, likely stimulated by the high concentrations of bioactive compounds in the plants serving as natural immune-modulators, stimulating macrophages and lymphoid tissues to synthesize immunoglobulins without triggering an energy-depleting inflammatory response [29]. Red blood cell and pack cell volume was within the normal range 7.00 – 12.00 (106/L) and 28.00 – 37.00 % cited by [32]. Mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentrations were within 30.00 – 40.00 fl, 17.00 – 25.00 pg and 32.00 – 60.00 g/dL referenced by [33].

Whereas total protein, albumin and globulin concentrations were higher ($p < 0.05$) for T3-T5 than for T2 and T1, other serum parameters were similar ($p > 0.05$) among the diets (Table 6). However, all values obtained in this study was within the normal reference range for healthy birds reported by [34]. Total protein values recorded in this study suggests that the experimental diet is adequate in protein necessary for the

growth of birds [27]. The total protein value recorded in this study is within 30.0 – 75.00 g/L cited by [35]. The non-significant difference ($p > 0.05$) in creatinine, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase concentrations is clear a evidence that these natural plants are entirely non-toxic. The liver and kidneys processed these compounds at a 10 g/kg inclusion rate without experiencing any physiological stress or toxicity, validating their safety for commercial poultry formulation [30]. The outcome obtained in this study is in agreement with the reports of [29,36].

Parameters	Quantity
Maize	50.00
Wheat bran	2.80
Groundnut cake	10.00
Soybean meal	25.00
Fish meal	5.00
Limestone	2.00
Bone meal	4.00
Salt	0.35
Lysine	0.30
Methionine	0.30
Vitamin premix	0.25
Total	100.0
Chemical composition	
Crude protein	23.21
Crude fibre	3.40
Ether extract	4.52
Calcium	1.18
Phosphorus	0.61
Metabolisabe energy (MJ/kg DM)	11.93

Table1: Ingredient and chemical composition of the experimental diets (% DM)

Compounds (mg/g)	<i>Bauhinia forficata</i> leaf powder	<i>Caulophyllum thalictroides</i> leaf powder	<i>Combretum micranthum</i> leaf powder
Phenol	209.1	189.3	120.0
Flavonoids	186.0	105.0	76.30
Tannins	72.24	54.20	60.10
Alkaloids	24.60	30.30	21.80
Steroids	84.80	72.20	45.70
Saponins	69.10	68.40	27.20

Table2: Phytochemical composition different medicinal plants

Parameters	T1	T2	T3	T4	T5	SEM
Number of birds	100	100	100	100	100	-
Duration of the experiment	28	28	28	28	28	-
Initial body weight (g/b)	55.36	55.12	55.18	55.11	55.08	0.02
Final body weight (g/b)	983.2 ^c	1200.5 ^b	1610.4 ^a	1618.3 ^a	1631.7 ^a	65.87
Body weight gain (g/b)	927.84 ^c	1145.4 ^b	1555.2 ^a	1563.1 ^a	1576.6 ^a	60.09
Average daily weight gain (g/b)	33.13 ^c	40.91 ^b	55.54 ^a	55.83 ^a	56.31 ^a	0.04
Cumulative feed intake (g/b)	1925.2 ^c	2109.3 ^b	2302.1 ^a	2311.8 ^a	2320.3 ^a	89.56
Average daily feed intake (g/b)	68.75 ^c	75.33 ^b	82.22 ^a	82.56 ^a	82.87 ^a	0.06
Feed conversion ratio	2.07 ^a	1.84 ^b	1.50 ^c	1.50 ^c	1.50 ^c	0.001

Note on Superscripts: ^{a, b, c, d} Means along the same row with different superscripts are significantly different (p<0.05). Rows without superscripts show no significant difference (p>0.05).

Treatment 1 (T1): Negative Control; received basal diet only (no additives).

Treatment 2 (T2): Positive Control; received basal diet supplemented with 10 g neomycin per kg of diet.

Treatment 3 (T3): Received basal diet supplemented with Bauhinia forficata leaf powder at 10 g/kg of diet.

Treatment 4 (T4): Received basal diet supplemented with Caulophyllum thalictroides leaf powder at 10 g/kg of diet.

Treatment 5 (T5): Received basal diet supplemented with Combretum micranthum leaf powder at 10 g/kg of diet.

Table3: effect of plant extract supplementation on the growth performance of Hubbard broilers (0 – 28 d)

Parameters	T1	T2	T3	T4	T5	SEM
Live weight	961.2 ^c	1280.6 ^b	1774.3 ^a	1778.8 ^a	1781.4 ^a	47.12
Dressed weight	710.0 ^c	1095 ^b	1506.1 ^a	1509.6 ^a	1512.7 ^a	40.08
Eviscerated weight	591.2 ^c	889.3 ^b	1345.3 ^a	1350.9 ^a	1358.1 ^a	38.50
Dressing percentage (%)	61.51 ^c	69.44 ^b	75.82 ^a	75.94 ^a	76.24 ^a	0.07
Head (g)	19.44 ^c	21.84 ^b	24.16 ^a	24.55 ^a	24.67 ^a	0.02
Neck (g)	28.46 ^c	31.35 ^b	38.05 ^a	38.16 ^a	38.58 ^a	0.03
Wing (g)	45.11 ^c	49.15 ^b	53.16 ^a	53.89 ^a	54.26 ^a	0.04
Back (g)	87.27 ^c	100.8 ^b	118.6 ^a	119.4 ^a	112.5 ^a	2.35
Breast (g)	98.46 ^c	112.8 ^b	125.9 ^a	126.5 ^a	127.3 ^a	3.76
Drumstick (g)	47.45 ^c	56.01 ^b	68.22 ^a	69.12 ^a	69.48 ^a	0.04
Thigh (g)	49.95 ^c	54.05 ^b	65.76 ^a	65.89 ^a	66.31 ^a	0.03
Shank (g)	22.86 ^c	27.11 ^b	30.24 ^a	30.55 ^a	30.87 ^a	0.02

Note on Superscripts: ^{a, b, c, d} Means along the same row with different superscripts are significantly different (p<0.05). Rows without superscripts show no significant difference (p>0.05).

Treatment 1 (T1): Negative Control; received basal diet only (no additives).

Treatment 2 (T2): Positive Control; received basal diet supplemented with 10 g neomycin per kg of diet.

Treatment 3 (T3): Received basal diet supplemented with Bauhinia forficata leaf powder at 10 g/kg of diet.

Treatment 4 (T4): Received basal diet supplemented with Caulophyllum thalictroides leaf powder at 10 g/kg of diet.

Treatment 5 (T5): Received basal diet supplemented with Combretum micranthum leaf powder at 10 g/kg of diet.

Table4: effect of plant extract supplementation on the carcass characteristics of Hubbard broilers (0 – 28 d)

Parameters	T1	T2	T3	T4	T5	SEM
Pack cell volume (%)	29.82 ^c	30.93 ^b	33.87 ^a	34.05 ^a	34.15 ^a	0.03

Red blood cell (106/L)	7.51 ^c	9.45 ^b	11.56 ^a	11.72 ^a	11.86 ^a	0.01
Haemoglobin (g/dL)	9.77 ^c	11.45 ^b	13.47 ^a	13.86 ^a	13.91 ^a	0.01
Mean corpuscular volume (fl)	30.08 ^c	34.01 ^b	37.71 ^a	38.62 ^a	38.95 ^a	0.04
Mean corpuscular haemoglobin (pg)	18.21 ^c	23.86 ^b	29.08 ^a	29.16 ^a	29.22 ^a	0.03
MCHC (g/dL)	33.75 ^c	43.21 ^b	48.86 ^a	48.95 ^a	48.98 ^a	0.05
White blood cell (109/L)	2.89 ^c	3.82 ^b	4.08 ^a	4.12 ^a	4.15 ^a	0.01
Lymphocytes (109/L)	2.28 ^c	3.00 ^b	3.66 ^a	3.70 ^a	3.72 ^a	0.01
Monocytes (109/L)	0.03 ^c	0.05 ^b	0.07 ^a	0.08 ^a	0.08 ^a	0.001

Note on Superscripts: ^{a, b, c, d} Means along the same row with different superscripts are significantly different (p<0.05). Rows without superscripts show no significant difference (p>0.05).

Treatment 1 (T1): Negative Control; received basal diet only (no additives).

Treatment 2 (T2): Positive Control; received basal diet supplemented with 10 g neomycin per kg of diet.

Treatment 3 (T3): Received basal diet supplemented with *Bauhinia forficata* leaf powder at 10 g/kg of diet.

Treatment 4 (T4): Received basal diet supplemented with *Caulophyllum thalictroides* leaf powder at 10 g/kg of diet.

Treatment 5 (T5): Received basal diet supplemented with *Combretum micranthum* leaf powder at 10 g/kg of diet.

Table5: effect of plant extract supplementation on the hematological indices of Hubbard broilers (0 – 28 d)

Parameters	T1	T2	T3	T4	T5	SEM
Total protein (g/L)	36.87 ^a	44.27 ^a	50.34 ^a	51.97 ^a	52.02 ^a	0.09
Albumin (g/L)	17.76 ^a	20.8 ^a	24.17 ^a	25.09 ^a	25.11 ^a	0.02
Globulin (g/L)	19.11 ^a	23.47 ^a	26.17 ^a	26.88 ^a	26.91 ^a	0.03
Creatinine (μmol/L)	32.12	33.85	33.93	34.01	34.05	0.03
Aspartate Aminotransferase (U/L)	66.24	69.12	69.38	69.43	69.71	0.07
Alanine Aminotransferase (U/L)	38.21	38.65	38.78	39.02	39.18	0.04
Alkaline phosphatase (U/L)	20.16	21.23	21.97	22.45	23.53	0.03

Note on Superscripts: ^{a, b, c, d} Means along the same row with different superscripts are significantly different (p<0.05). Rows without superscripts show no significant difference (p>0.05).

Treatment 1 (T1): Negative Control; received basal diet only (no additives).

Treatment 2 (T2): Positive Control; received basal diet supplemented with 10 g neomycin per kg of diet.

Treatment 3 (T3): Received basal diet supplemented with *Bauhinia forficata* leaf powder at 10 g/kg of diet.

Treatment 4 (T4): Received basal diet supplemented with *Caulophyllum thalictroides* leaf powder at 10 g/kg of diet.

Treatment 5 (T5): Received basal diet supplemented with *Combretum micranthum* leaf powder at 10 g/kg of diet.

Table6: effect of plant extract supplementation on serum biochemical indices of Hubbard broilers (0 – 28 d)

Conclusion

The dietary inclusion of *Bauhinia forficata*, *Caulophyllum thalictroides*, and *Combretum micranthum* leaf powders at 10 g/kg represents a highly viable, safe, and superior alternative to synthetic antibiotics like neomycin. Among the three, *Bauhinia forficata* (T3) emerges as a particularly potent candidate due to its exceptionally high load of flavonoids and phenols. Overall, these phytogenics successfully stimulate appetite, optimize gut architecture for superior FCR, support robust (yet safe) hematological profiles, and ultimately maximize the yield of high-value carcass cuts in Hubbard broilers.

References

1. Bashir, Musa, John O. Alagbe, Adegbite Motunrade Betty, and E. A. Omokore. "Growth Performance, caeca microbial population and immune response of starter broiler chicks fed aqueous extract of *Balanites aegyptiaca* and *Alchornea cordifolia* stem bark mixture." *United Journal for Research and Technology* 2, no. 2 (2020): 13-21.

2. Alagbe, J.O., Shittu, M.D., Ramalan, S.N., Tanimomo, K.B and Adekunle, D.A. (2022). Growth performance, semen quality characteristics and hormonal profile of male rabbit bucks fed Rubia cordifolia root extracts. *International Journal of Biological Engineering and Agriculture* 1(1): 1-13.
3. Alagbe, J.O (2025). Effect of Partial Replacement of Soyameal with Albizia lebbeck seed meal on the growth performance, egg quality and haemato-biochemical constituents of Nera black hens. *Discovery Agriculture*, 11(24), e18da3161
4. Anaso, Emmanuel, Alagbe John, Abdulbasit Aderibigbe, Joy Anaso, and Emeka Fidelis. "Dietary Inclusion of Ginger (Zingiber Officinale) Essential Oil as a Sustainable Feeding Strategy: Effects on Carcass Characteristics, Meat Fatty Acid Composition, and Quality Attributes in Holstein Friesian× White Fulani Bulls." *International Journal of Agriculture Environment and Food Sciences* 9, no. 4 (2025): 1361-1373.
5. Emmanuel, U.A and Alagbe, J.O. (2026). Nutrient digestibility, rumen fermentation and Nitrogen retention of Yankasa rams supplemented Piliostigma thonningii essential oil based diet. *Research in: Agricultural and Veterinary Sciences*, 9(3): 106-120.
6. Emmanuel, U.A and Alagbe, J.O. (2026). Ecofriendly feeding strategies: effect of ginger essential oil on carcass yield and meat quality in white Fulani bulls. *Research in: Agricultural and Veterinary Sciences*, 9(3): 169-180.
7. Zubairu, H., Mubarak A. Adediran, and J. O. Alagbe. "Effect of Partial Replacement of Soya Meal with Sphenocentrum Jollyanum Seed Meal on the Growth Performance and Haematological Constituents of Broiler Chicken." *International Journal of Integrative Research* 3, no. 10 (2025): 741-752.
8. Alagbe, Olujimi John. "Effects of Feeding Different Dose of Cynoglossum Zeylanicum Leaf Extract on the Growth Performance and Haemato-Biochemical Parameters of Pekin Ducks." *Agricultural Development* 10, no. 2 (2025): 1-6.
9. Alagbe, J.O (2025). Effect of feeding different levels of Terminalia arjuna stem bark extract on the haematological and serum biochemical parameters of Guinea fowl. *Clinical Investigation and Research*, 3(1); 10.58489/3066-4896/003.
10. Alagbe, Olujimi John. "Effects of Dietary Supplementation of Date Palm (Phoenix dactylifera) on the Growth Performance, Carcass Characteristics, and Immune Response of Broiler Chicken." *Farm Animal Health and Nutrition* 3, no. 4 (2024): 64-71.
11. Sharma, Singh, Alagbe Olujimi John, Liu Xing, Sharma Ram, and Kumar Amita. "Comparative analysis of ethanolic Juniperus thurifera leaf, stem bark and root extract using gas chromatography and mass spectroemetry." *International Journal of Agriculture and Animal Production* 2, no. 6 (2022): 18-27.
12. Anorue, Daniel Nnadozie, Friday Ubong, and Alagbe Olujimi John. "Investigating the effects of pawpaw (Carica papaya) essential oil dietary supplementation on the growth performance and carcass characteristics of broilers." *Research in: Agricultural and Veterinary Sciences* 7, no. 3 (2023): 164-174.
13. Shittu, M.D., Alagbe, J.O., Alaba, O., Okanlawon, E.O., Ad-elakun, F.A., Emmanuel, E.O and Adejumo, D.O. (2024). Effect of ginger, garlic and Negro pepper on the gut microbes, gut histomorphometry and pathological assessment of selected organs of broiler chickens. *Association of Deans of Agriculture in Nigerian Universities*, 5: 105-121.
14. Alagbe, J.O. (2025). Investigating the effect of dietary supplementation of Ficus exasperata oil on the growth performance, apparent digestibility and nitrogen utilization of weaner rabbits. *International Journal of Applied and Advanced Multidisciplinary Research*, 3(9): 661-668.
15. Morris, Hernández, and Alagbe John Olujimi. "INFLUENCE OF Odontonema Strictum OIL ON THE GROWTH PERFORMANCE AND RUMINAL FERMENTATION OF BARBARI BUCKS." *Research in: Agricultural & Veterinary Sciences* 9, no. 2 (2025).
16. National Research Council, and Subcommittee on Poultry Nutrition. Nutrient requirements of poultry: 1994. National Academies Press, 1994.
17. Hernandez, M and Alagbe, J.O. (2025). Influence of Abrus procatorious crude oil supplementation on the growth performance, Nutrient digestibility, Ruminal fermentation and Microbial population of Malabari Bucks. *International Journal of Global Sustainable Research*, 3(7): 527-538.
18. Alagbe, J.O. (2020). Performance, hematology and serum biochemical parameters of weaner rabbits fed different levels of fermented Lagenaria brevifera whole fruit extract. *Advances in Research and Reviews*, 2020, 1:5.
19. Alagbe, J. O., M. D. Shittu, T. K. Ojediran, E. A. Omokore, E. O. Oluwayinka, D. N. Anorue, S. Bamigboye, and E. Effiong. "Dietary supplementation of Ocotea aciphylla essential oil: effects on growth performance and nutrient digestibility of weaned pigs." *World Journal of Agriculture and Forestry Sciences* 2, no. 2 (2024): 43-49.
20. Omokore, E. O., and J. O. Alagbe. "Efficacy of dried Phyllanthus amarus leaf meal as an herbal feed additive on the growth performance, haematology and serum biochemistry of growing rabbits." *International Journal of Academic Research and Development* 4, no. 3 (2019): 97-104.
21. Zhao, L.-J., Liu, W., Xiong, S.-H., Tang, J., Lou, Z.-H., Xie, M.-X., ... Liao, D.-F. (2018). Determination of total flavonoids contents and antioxidant activity of Ginkgo biloba leaf by near-infrared reflectance method. *International Journal of Analytical Chemistry*, 2018, 1-7. DOI: <https://doi.org/10.1155/2018/8195784>
22. Abdelli, Nedra, David Solà-Oriol, and José Francisco Pérez. "Phytogetic feed additives in poultry: achievements, prospective and challenges." *Animals* 11, no. 12 (2021): 3471.
23. Ayodele, S. O., O. D. Oloruntola, and J. O. Agbede. "Effect of Alchornea cordifolia leaf meal inclusion and enzyme supplementation on performance and digestibility of rabbits." *World Rabbit Science* 24, no. 3 (2016): 201-206.

24. Oloruntola, Olugbenga D., Simeon O. Ayodele, Deborah A. Oloruntola, Olumuyiwa J. Olarotimi, Andrew B. Falowo, Victor O. Akinduro, Francis A. Gbore, Olufemi A. Adu, and Johnson O. Agbede. "Dietary supplementation of Capsicum powder affects the growth, immunoglobulins, pro-inflammatory cytokines, adipokines, meat, and liver histology of aflatoxin B1 exposed broiler chickens." *Toxicon* 240 (2024): 107640.
25. Gilani, Syed Muddassar Hussain, Sitwat Zehra, Saddia Galani, and Asma Ashraf. "Effect of natural growth promoters on immunity, and biochemical and haematological parameters of broiler chickens." *Tropical Journal of Pharmaceutical Research* 17, no. 4 (2018): 627-633.
26. Oloruntola, Olugbenga David, Johnson Oluwasola Agbede, Simeon Olugbenga Ayodele, and Deborah Adebukola Oloruntola. "Neem, pawpaw and bamboo leaf meal dietary supplementation in broiler chickens: Effect on performance and health status." *Journal of Food Biochemistry* 43, no. 2 (2019): e12723.
27. Ijadunola, T. I., M. A. Popoola, M. O. Bolarinwa, K. A. Ayangbola, and C. A. Omole. "Effects of supplemental Vitamins E and C on growth performance and physiological responses of broiler chicken under environmental heat stress." *Nigerian Journal of Animal Science* 22, no. 3 (2020): 17-25.
28. Oloruntola, Olugbenga David, Samuel Adebowale Adeyeye, Olumuyiwa Joseph Olarotimi, Victor Olabisi Akinduro, Olufemi Adesanya Adu, Francis Ayodeji Gbore, and Ojurreoluwa Adebimpe Ayodele. "Dietary supplementation with mango leaf powder influences broiler chickens' growth characteristics, blood parameters, and carcass." *Acta Scientiarum. Animal Sciences* 47 (2025): e71190.
29. Orlowski, Sara, Joshua Flees, Elizabeth S. Greene, Danielle Ashley, Sun-Ok Lee, Famous L. Yang, Casey M. Owens, Michael Kidd, Nicholas Anthony, and Sami Dridi. "Effects of phyto-genic additives on meat quality traits in broiler chickens." *Journal of Animal Science* 96, no. 9 (2018): 3757-3767.
30. Islam, M. S., Lucky, N. S., Islam, M. R., Ahad, A., Das, B. R., Rahman, M. M and Siddivi, M. S. I. (2004). Haematological parameters of Fayoumi, Assil and local chickens reared in Sylhet Region in Bangladesh. *International Journal of Poultry Science*, 4(10), 748-756.
31. Adewale, A.O., Alagbe, J.O and Adekemi, G.A (2021). Dietary supplementation of Rauvolfia vomitoria root extract as a phyto-genic feed additive in growing rabbit's diets: Haematology and Serum biochemical indices. *International Journal of Orange Technologies* 3(3): 31-42.
32. Alagbe Olujimi John, Daniel Nnadozie Anuore, Shittu Muritala Daniel, Emiola Adewale, Akande Taiwo and Adegoke Adegbite Emmanuel (2023). Impact of dietary supplementation of Carica papaya essential oil on the blood chemistry of broiler chickens. *Science Letters*, 11(3): 105 - 110.
33. Muritala, Daniel Shittu., Alagbe, J.O., Ojebiyi, O.O., Ojediran, T.K and Rafiu, T.A. (2022). Growth performance and haematological and serum biochemical parameters of broiler chickens given varied concentrations of Polyalthia longifolia leaf extract in place of conventional antibiotics. *Animal Science and Genetics* 18(2): 57-71.
34. American Metabolic Testing Laboratories, Inc. Chemistry Profile. (2001). www.caprofile.net/t3.html.
35. Mitruka, H. M. & Rawnsley, S. K. (1977). Chemical, Biochemical and Haematological Reference in Normal Experimental Animals. Mason, N.Y., pp. 287-380.
36. Café, Marcos Barcellos, Fabrício Pereira Rinaldi, Hugo Ribeiro Morais, M. R. B. M. Nascimento, Antônio Vicente Mundim, and Cristiane Ferreira Prazeres Marchini. "Biochemical blood parameters of broilers at different ages under thermoneutral environment." *Worlds Poultry Science Journal* 5, no. 9 (2012): 143-146.

Citation: Kumar, Amsar, Alagbe, John Olujimi, Babatunde Tanimomo, Rufus, Adebisi Oluwafemi. Evaluation of *Bauhinia forficata*, *Caulophyllum thalictroides*, and *Combretum micranthum* Leaf Powders as Alternatives to Antibiotic Growth Promoters in Broiler Diets. *Arch. Immunol. Res. Ther.* Vol. 5 Iss. 2. (2026) DOI: 10.58489/2836-5003/019