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## UPSHOT OF PLANT SUPPLEMENTS ON BLOOD INDICES AND NEUTROPHILS ADHESION POTENTIAL IN BROILER CHICKS

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### ABSTRACT

Immune support by botanical or natural supplement plays vital roles against microbial infections and abnormal functioning of cells and organs in the body system. It has been established that the complex, integrated immune system needs multiple specific micro nutrients which play vital, often synergistic roles in every stage of the immune response. This study evaluated the effects of plant supplements on blood indices and neutrophil adhesion potential in broiler chicks. Extracts from *Azadirachta indica* (Neem), *Curcuma longa* (turmeric), and *Allium sativum* (garlic) were combined with vitamin C and administered to 3-week-old broiler chicks. After 11 days, blood samples were analyzed, revealing non-significant increases in lymphocytes and neutrophils, with the highest values observed in the *Baphia nitida* leaf extract (BN) group (62.14% lymphocytes and 35.56% neutrophils) compared to the control group (56.80% lymphocytes and 30.50% neutrophils). Notably, the plant supplements significantly enhanced neutrophil adhesion potential, with BN recording the highest value (26.32%) compared to the control group (14.93%). The results suggest that these plant supplements, particularly BN, may have immunomodulatory effects in broiler chicks, enhancing neutrophil adhesion and lymphocyte counts.

Keywords: Plant supplements, Broiler chicks, Blood indices, Neutrophil adhesion Immunomodulation

## INTRODUCTION

Immune system protects our body from the attack of foreign bodies in the same way as our soldiers protect our country. It always remains in a proactive state with an ability to distinguish “non-self” from “self.” There is no doubt that people with immune responses below the “normal” level are more susceptible to infectious disease. Nutrition status is a very significant factor in promoting immune competence. It is important in synthesis and secretion of signaling molecules, free radical generation, cell proliferation, and the function connected with immune suppression (Abdel-Ghaffer *et al.*, 2012). Under nutrition or deficiency of any nutrients can disturb the activities of our immune system and make our body prone to different diseases. Consumption of a balanced diet (a diet that contains right amount of all the nutrients) is therefore essential for the proper functioning of our immune system (Emadi *et al.*, 2009).

Several research studies have revealed that there is direct correlation between increased consumption of fruits and reduced risk of chronic ailments. Various biological reasons have been reported to suggest that intake of fruits could prevent occurrence of chronic ailments. One of the key reasons is that

fruits contain a wide range of health beneficial components such as vitamins, minerals, and phytochemicals. It has been demonstrated that after vaccination antibody count is higher in people who include a good amount of fruits and vegetables in diet. It is so because of the presence of various antioxidants in fruits and vegetables that scavenges harmful reactive oxygen species (ROS) that are found in higher amounts during old age. Apart from it, antioxidants enhance both the natural (antigen-independent) and acquired (antigen-specific) immune response. Particularly, antioxidant molecules can hold-up both “weapons” of acquired immunity, B and T lymphocytes (Ponnuswamy *et al.*, 2011). Reduced levels of antioxidants are associated with impairment of immune system and more susceptibility to infectious diseases. Various phytochemicals, vitamins, and minerals such as flavonoids, quercetin, kaempferol, ellagic acid, catechins, ascorbic acid, folic acid, zinc, and selenium are present in fruits, which protect our body from the harmful effects of ROS. Evidence from in vitro studies have revealed their immunomodulatory, anti-inflammatory, anti-microbial, and radical scavenging activity. They also help in the smooth functioning of various cells of adaptive and innate immune system. Thus, their

proper intake can enhance the immune system and resistance to infection (Meng *et al.*, 2018). It is crucial that scientific proofs related to antioxidative, anti-inflammatory, and immunomodulation benefits of fruits need to be examined carefully and objective information should be made available to scientific minds. Until now different studies have been done to provide information about the immunomodulatory activity of bioactive compounds in fruits. However, there was an absence of a comprehensive review that includes different commonly consumed fruits and the role played by phytochemicals in strengthening specific part of the immune system along with its mechanism. In this review paper, we tried to present the immunity-boosting effects of various bioactive constituents present in fruits and highlight their essential role in protecting us from the invasion of various microorganisms. It might be useful for future researchers while planning strategies like selecting effective bioactive ingredients to prepare functional foods with immune-enhancing potential (Rasheed *et al.*, 2016). Infectious bursal disease (IBD) also known as gumboro disease and infectious bursitis is an acute, highly contagious viral disease of young (immature) chickens (three to five weeks old) caused by the infectious bursal

disease virus belonging to the genus Avibirnavirus (birnavirus) an RNA virus of the family Birnaviridae (Hossain *et al.*, 2011). The virus is a non-enveloped icosahedral, bisegmented double stranded (dsRNA) virus with a diameter of about 55-60 nm. The disease was first discovered in 1957 in Gumboro county, Delaware, USA by Cosgrove as a result, it is often referred to as Gumboro. Since its first appearance in 1962 in Gumboro county USA, IBD has been reported in the poultry industries and in concentrated poultry production areas all over the world causing devastating outbreaks (Sarmiento *et al.*, 2011). The disease is particularly important due to high mortalities, lowered productivity among infected chickens. It is of high economic importance in Nigeria as it results in tremendous loss to poultry farmers in terms of mortality and immunosuppression. Outbreak of IBD has been reported in IBD vaccinated flocks. Suboptimal humoral immune response to IBD vaccination has been observed in birds. Research has therefore been targeted at improving the immunogenicity of some vaccines by using antioxidants such as Vitamin C. (Rasheed *et al.*, 2016). Reports on their effectiveness or otherwise are conflicting and therefore inconclusive, possibly due to difference in the

virulence of the virus strains or weather conditions. Researches into natural products for solving health problems have been encouraged by the World Health Organisation and Food and Agricultural Organisation. In ancient immemorial period Neem has been used as a disincentive agent against highly contagious smallpox and other infectious diseases and was also regarded to defend against evil spirits from time. In the Indus civilization, the use of Neem tree is as old as 4,500 years during the period of Harappa culture (one among the great civilisation in the world). Centre for Traditional Medicine and Research (CTMR), Chennai, India, revealed the medicinal uses of different parts viz, fruits, seeds, leaves, roots, bark etc., of neem trees. It explains use of neem flower against bile disorders, neem leaves to prevent and treat ulcers and neem bark to brawl against paralysis and CNS disorders. Old evidences obtained from two great civilizations Harappa and Mohenjo-Daro of ancient world also witnessed that *A. indica* was the prominent herb of therapeutic importance at that time not only in Indian context but in world as well. According to epic of Mahabharata, Nakul and Sahadeva used Neem oils for treatment of wounds in horses and elephants (Emadi *et al.*, 2009). This research is aimed at evaluating the

upshot of plant supplements on blood indices and neutrophils adhesion of broiler chicks.

## **MATERIALS AND METHODS**

### **Purification of the Isolates**

The plate that showed discrete colonies were selected after 24 - 48 h and each colony was aseptically streaked using a sterile wire loop on a sterile poured plate (90mm x 15mm) containing nutrient agar (BIOTECH) prepared according to the manufacturers description. after which it was incubated at their required growth conditions (Iheukwumere and Iheukwumere, 2022c; Iheukwumere *et al.*, 2022d; and Iheukwumere and Iheukwumere, 2022e).

### **Characterization of the Bacteria Pure Isolates**

The pure isolates were characterized using the morphological, biochemical and molecular characteristics as described by Iheukwumere *et al.* (2018), Iheukwumere *et al.* (2022f), Iheukwumere *et al.* (2023a) and Iheukwumere *et al.* (2023b).

### **Morphological characteristics of the Bacteria isolates**

The cultural descriptions (size, appearance, edge, elevation, colour) of the isolates were carried out as described

in Iheukwumere *et al.* (2024) and Iheukwumere *et al.* (2022g). The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Obianom *et al.*, (2024), Egbe *et al.* (2025a) and Manasseh *et al.* (2025). The presence or absence of capsule was also carried out as described by Ekechukwu *et al.* (2025a). The presence or absence of flagellum was determined by carrying out motility test as described by Ekechukwu *et al.* (2025b).

### **Gram staining technique**

A thin smear was made in a cleaned grease free microscopic slide (75mm×25mm), air dried heat fixed. The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with cleaned water. Gram iodine solution (0.01%) was then applied and allowed for 60 seconds. This was rinsed with cleaned water. This was followed by decolourizing the slide content with 95%w/v ethyl alcohol for 10seconds and then rinsed with cleaned water. The smear was then counter stained with safranin solution (0.025%) for 60 seconds, rinsed with cleaned water, blot drained and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope

using × 100 objective lens as described by Ekechukwu *et al.* (2025c), Egbe *et al.* (2025b) and Egbe *et al.* (2025c).

**Motility test:** This was done using the method described by Iheukwumere *et al.* (2025a), Iheukwumere *et al.* (2025b) and Iheukwumere *et al.* (2025c). A semi-solid medium prepared by mixing 5.0g of bacteriological agar (BIOTECH) with 2.0g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10 ml portion in different test tubes. The test tubes were allowed to set in vertical positions and then inoculate the test organisms by performing a single stab down the centre of the test tube to half the depth of the medium using sterile stabbing needle. The test tubes were kept in an incubator in vertical position at  $35\pm 2^{\circ}\text{C}$  for 24h.T

### **Biochemical characteristics of the isolates**

**Indole test:** Indole is a nitrogen containing compound formed when the amino acid tryptophan is hydrolyzed by bacteria that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. This was done using the method described by Iheukwumere *et al.* (2025d), Iheukwumere *et al.* (2025e) and Iheukwumere *et al.* (2025f).

The isolates were cultured in peptone water in 500.0 ml of deionized water. Ten millilitres of peptone water was dispensed into the test tubes and sterilized. The medium was then inoculated with the isolates and kept in an incubator at 37°C for 48 hr. Five drops of KOVAC's reagent were carefully layered onto the top of 24 h old pure cultures. The presence of indole was revealed by the development of red layer colouration on the top of the broth cultures.

**Sugar fermentation test:** The capability of the isolates to metabolize some sugars (glucose, xylose, ducitol, maltose, arabinose, inositol, mucate and lactose) with the resulting formation of acid and gas or either were carried out using sugar fermentation test. One litre of 1% (w/v) peptone water was added to 3 mL of 0.2% (w/v) bromocresol purple and 9 ml was dispensed in the test tube that contained inverted Durham tubes. The medium was then sterilized by autoclaving. The sugar solution were prepared at 10% (w/v) and sterilized. One milliliter of the sugar was dispensed aseptically into the test tubes as described by Dim *et al.* (2025a) and Dim *et al.* (2025b). The medium was then inoculated with the appropriate isolates and the cultures incubated at 37°C for 48 h and were examined for the

formation of acid and gas. Change in colour from purple to yellow indicated acid formation while gas formation was assessed by the presence of bubbles in the inverted

**Methyl red test:** Using the method described by Dim *et al.* (2025c), Iheukwumere *et al.* (2025g). The glucose phosphate broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37°C for 48 hr. After incubation, five drops of 0.4 % solution of alcoholic methyl red solution was added and mixed thoroughly, and the result was read immediately. Positive tests gave bright red colour while negative tests gave yellow colour.

**Voges-Proskauer test:** Using the method described by Iheukwumere *et al.* (2025h), Ike *et al.* (2025a). The glucose phosphate broth was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37°C for 48hr. After incubation, 1.0 mL of 40% potassium hydroxide (KOH) containing 0.3% Creatine and 3 ml of 5% solution of  $\alpha$ -naphthol was added in the absolute alcohol. Positive reaction was observed by the development of pink

colour within five minutes.

**Citrate utilization test:** The Simmon's Citrate Agar was prepared according to the manufacturer's direction and the isolates were inoculated by stabbing directly at the center of the medium in the test tubes and incubated at 37°C for 48 hr. Positive test was shown by the appearance of growth with blue colour, while negative test showed no growth and the original green colour was retained as described by Ike *et al.* (2025b) and Ike *et al.* (2025c).

**Catalase test:** The test was carried out as described by Ike *et al.* (2025d) and Ike *et al.* (2025e). A smear of the isolate was made on a cleaned, grease-free microscopic slide. Then, a drop of 30% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was added on the smear. Prompt effervescence indicated catalase production.

**Oxidase test:** The test was carried out using the method described by Ugwu *et al.* (2025a). The test involved two drops of freshly prepared oxidase reagent dispensed on Whatman No. 1 filter paper which was placed in Petri dish, and a smear of the test isolate was made on the spot using a sterile stick. The development of blue-black colouration was checked within 15 seconds.

**Urease test:** This was carried out as described by Ugwu *et al.* (2025b). The

urea agar slant was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into sterilized medium. This was incubated at 37°C for 48 h. After incubation, observation was made for the presence of purple-pink colouration.

### **Molecular characterization of the isolates**

#### **Extraction and purification of DNA:**

All strains were plated on Nutrient Agar (Biotech) and incubated at 37°C for 24 hr. Using the Zymo Research (ZR) DNA miniprep™ kit (Category No. D6005; Irvine, California, USA), bacterial genomic DNA was extracted and purified as described by Iheukwumere *et al.* (2018), Iheukwumere *et al.* (2020) with the procedures outlined in the kit.

#### **Determination of the quality of**

**extracted DNA:** Using mass spectrophotometer (Nanodrop), One micro litre (1µL) was aseptically dropped into a fresh space in the chamber and the chamber was lightly closed which was then linked to a computer system which showed the window that discovered the value of the sample at 260/280nm as described by (Iheukwumere *et al.*, 2017a; Chude *et al.*, 2020).

#### **Amplification of DNA and gel**

**electrophoresis of PCR product:** This was analysed using Master cycler Nexus Gradient (Eppendorf). A mixture of primer (20 µL), template DNA (20µL), water (72 µL) and master mix (108 µL), which comprises taq polymerase, dimethylsulfoxide (DMSO), magnesium chloride (MgCl<sub>2</sub>) and nucleotides triphosphates (NdTPs), was made in 1.5 mL tube and homogenized using vortex mixer (Eppendorf). This was then positioned in the block chamber of the master cycler and then programmed. The PCR program for conditions were as follows: initial incubation at 94°C for 5 mins, followed by 35 cycles of denaturation at 94°C for 15 secs, annealing at 55°C for 15 secs, elongation at 72°C for 21 secs and final extension period for 10 mins at 72°C. The amplified products were electrophorezed in 1.0% agarose gel and a1kb DNA ladder was used as a size reference. After staining with 3µL of nucleic acid stain (GR green), the gel was documented with gel documentation apparatus (Iheukwumere *et al.*, 2017b; Iheukwumere *et al.*, 2017c; and Iheukwumere *et al.*, 2018b).

**DNA sequencing of 16s rRNA fragment:** The 16S rRNA amplified PCR products generated from universal primer (16S), was used for the sequencing using ABI DNA sequencer

(Applied Biosystem Inc) at International Institute of Tropical Agriculture (IITA), Ibadan using the method of Iheukwumere *et al.* (2017d), and Iheukwumere *et al.* (2018c).

**Computational Analysis:** This was analysed making use of the modified method of Iheukwumere *et al.* (2025i) and Iheukwumere *et al.* (2025j). The chromatograms generated from the sequences were cleaned to obtain regions with normal sequences. The cleaned nucleotides were aligned using pair wise alignment tool. The consensus sequences formed by the alignment of the forward and reverse sequences were used to perform the Basic Local *Alignment* Search Tool (BLAST) using National Centre for Biotechnology Information BLAST over the internet. The sequences of the isolates with 95% and above similarities were accepted. Also the maximum scores, total scores and accession numbers of the isolates were assessed. The relatedness of the isolates was determined by tracing their phylogenetic tree using DNA distance neighbour phylogenetic tree tool.

**Experimented Chicks:** A total of twenty four (24) broiler chicks (3 weeks old) were purchased from poultry market located at Ihiala market, Ihiala L. G. A. in Anambra State were used for the study. The chicks were kept in



separate, thoroughly cleaned and disinfected house and provided with feeds and water ad libitum. All the chicks were vaccinated against Newcastle disease using Lasota vaccine strains at 6 and 19 days of age, against infectious bronchitis using live H120 strain at 6 days old and also against avian influenza (A1) disease using inactivated H5N1 virus vaccine strain at 7 days old. All the vaccines were given via eye drop instillation except (A1) vaccine which was given through subcutaneous route at the back of the neck from the folder report collected from the poultry farmer.

**Preparations of Plant Materials:** The leaves of *Azadirachta indica*, (Neem plant) leaves of *Baphia nitida*, rhizomes of *Allium sativum* (garlic) and roots of *Curcuma longa* were collected from Onitsha, Anambra State, Nigeria. The plant material was authenticated appropriately Dr B. Garuba, in Botany Department, Michael Okpara Federal University of Agriculture, Umudike. The plant material was washed and dried under shade at room temperature for 14 days. The dried plant material was ground to powder form using sterile electric grinder (Iheukwumere *et al.*, 2020; Ejike *et al.* 2017; Nwobodo *et al.*, 2018; and Ekesiobi *et al.*, 2025).

**Extraction Procedure:** A 2000 mL Soxhlet extractor that has three main sections: a percolator (boiler and reflux) which circulates the solvent, a thimble (usually made of thick filter paper) which retains the solid to be extracted, and a siphon mechanism, which periodically empties the thimble was used for process. Twenty grams (100 g) of the plant material to be extracted was placed inside the thimble. The thimble was then loaded into the main chamber of the Soxhlet extractor. Then 1000 mL of ethanol was placed in a 1000 mL distillation flask. The flask was placed on the heating mantle (2000 mL, 220 V, 500 W). The Soxhlet extractor was placed at the top of the flask. A reflux condenser was placed at the top of the extractor. When the ethanol was heated to reflux, the solvent vapour travelled up a distillation arm, and flooded into the chamber housing the thimble of solid. The condenser ensured that any solvent vapour cooled, and dripped back down into the chamber housing the solid material. The chamber containing the solid material slowly filled with warm solvent. When the Soxhlet chamber was almost full, the chamber was emptied by the siphon. The solvent then returned to the distillation flask. The thimble ensured that the rapid motion of the solvent did not transport any solid material to the still pot. This cycle was

allowed to repeat many times for 12 h. After extraction, the solvent is removed, typically by means of a rotary evaporator to collect the extract.

**Preparation of Extracts:** The plant extracts were each reconstituted with phosphate buffer saline (PBS). One (1.0) g of the ethanolic plant extracts were each dissolved in 10 ml of PBS to make 0.10 ppm of the extracts using sterile conical flasks. This was evenly homogenized and stored in clean sterile containers for use (Iheukwumere *et al.*, 2020; Iheukwumere *et al.*, 2025k; Iheukwumere *et al.*, 2025l).

**Preparation of Plant Supplements:** A 50 mL portion of the prepared extract (100 mg/mL or 0.10 ppm) was carefully mixed 50 mL portion of vitamin C (100 mg/mL or 0.10 ppm) in order to form 100 mL portion of the respective solution of NeemVic (NE), TumeriVic (TU) and GarliVic (GA).

**Antigen preparation:** This was carried out using the method described and published by Nfambi *et al.* (2015). Fresh blood sample was collected from healthy sheep from Uli in Ihiala L. G. A., Anambra State, and this was mixed with sterile Alsever's solution (1:1). The sample was centrifuged at 2000 xg for 5 min to enable the red blood cells (RBCs) settled at the bottom of the test tube.

Then the supernatant was discarded and the sediment was collected as the sheep red blood cells (SRBCS). The SRBC was then washed three times with pyrogen- free phosphate buffered saline (PH 7.2). This was then kept under refrigeration for the study.

**Experimental protocols for the *in vivo* models:** A total of 36 broiler chicks were used for this study. The broiler chicks were grouped into six groups, and each group comprises 6 chicks. . A 0.5 mL/100 g of *Baphia nitida* leaf extract (BN), GA, NE, and TU each was orally administered to each of group of broiler chicks, and the remaining group was giving only feed and water as control group. The blood indices and neutrophil adhesion potential were assessed from the blood samples drawn from the chicks after 11 days.

**Hematological Indices:** The blood samples collected from the broiler chicks were examined using Automated Hematology Analyzer (MIN DRAY BC – 360), and the variations in the red blood cells (RBCs), lymphocytes, monocytes, neutrophils, eosinophils and basophils were assessed and recorded as described in the work published by Agiang *et al.* (2017), Iheukwumere *et al.* (2022a), Iheukwumere and Iheukwumere (2022a).

**Determination of Neutrophil Adhesion:** On day 11, after administration of the plant supplements, blood samples were drawn from vein of the broiler chicks and analyzed for total white blood cell counts (WBC) and differential white blood cell counts (DWC) to obtain the percentage neutrophils. After the initial counts, the blood samples were incubated with 80 mg/mL of nylon fibres for 15 min at 37°C. The incubated blood samples were again analyzed for WBC and DWC. The following were calculated:

$$NI = WBC \times \% \text{ Neutrophil}$$

$$\text{Neutrophil Adhesion} = \frac{(NI_U - NI_t)}{NI_U} \times 100$$

NI = Neutrophil Index,  $NI_U$  = NI for untreated sample,  $NI_t$  = NI for treated sample

**Statistical Analysis:** The data obtained in this study were presented in tables and figures. Their percentages were also calculated. The sample means and standard deviations of some of the analytical data were also calculated. The significance of this study was determined at 95% using one way analysis of variance (ANOVA). Pairwise comparison was analyzed using student “t” test as described by Okeke *et al.* (2017), Iheukwumere *et al.* (2022b), Iheukwumere *et al.* (2017e), Nwike *et al.* (2017), Amadi *et al.* (2017), and Iheukwumere *et al.* (2025l).

## RESULTS

The study showed elevated in the value of white blood cells (WBC), Lymphocytes. Monocytes, Lymphocyte counts and neutrophil counts when compared to the control group. There was slight decrease in the PCV and RBC values. The values of Hb varied; there was an increase among chicks administered Baphia nitida extract while those administered tumeriViC, GarliViC, NeemViC and VitaminC showed slight decrease when compared to the control group. The percentage of neutrophils of neutrophils also showed slight decrease. There were variations in the values of basophils and eosinophils, and these variations were statistically non-significant ( $P>0.05$ ). Also, the increase in lymphocytes and WBC observed among the broiler

chicks administered *Baphia nitida* extract, tumeriViC, GarliViC, NeemiViC and Vitamin C were statistically non-significant ( $P>0.05$ ).

The percentages of neutrophil adhesion of the blood samples drawn from the experimented chicks are shown in Table. The neutrophil indices of untreated blood samples, and those broiler chicks administered *Baphia nitida* extract recorded the highest value, followed by TumeriViC, GarliViC, NeemViC and Vitamin C. Similar trends were recorded on treated blood samples. It was also observed that the neutrophil indices and neutrophil adhesion values of treated and untreated blood samples were higher than that of control group, and these were statistically significant ( $P<0.05$ ) among those broiler administered *Baphia nitida* extract and TumeriViC

Table1: Hematological indices of the blood samples drawn from the experimented chicks

Parameter	BN	TUM	GAR	NEE	VC	CON
PCV(%)	28.67	27.22	25.46	27.29	25.62	28.77
Hb(g/dL)	8.86	8.64	8.14	8.68	8.08	8.81
RBC( $\times 10^{12}$ cells/L)	5.69	5.14	4.82	5.21	4.91	5.71
WBC( $\times 10^9$ cells/L)	5.22	5.10	4.27	4.02	3.71	3;38
Lym(%)	62.14	61.86	59.41	58.27	57.70	56.80
Mon(%)	3.90	4.20	4.60	4.36	3.91	3.90
Neu(%)	30.56	31.18	33.60	34.92	34.71	35.50
Eos(%)	1.10	0.80	1.30	1.40	1.10	1.20
Bas(%)	0.2	0.2	0.5	0.2	0.3	0.3
Lym( $\times 10^3/\mu\text{L}$ )	8.02	8.11	6.82	6.47	5.82	5.76
Mon( $\times 10^3/\mu\text{L}$ )	0.26	0.29	0.34	0.37	0.31	0.30
Neu( $\times 10^3/\mu\text{L}$ )	5.11	5.07	4.76	4.41	4.38	4.25
Eos( $\times 10^3/\mu\text{L}$ )	0.40	0.35	0.50	0.55	0.40	0.45

Table 2: Neutrophils adhesion of blood samples drawn from the experimented broiler chicks

Sample	Untreated sample			Treated sample			
	WBC(X10 <sup>3</sup> c ells/MCL)	NEU	NI %	WBC(X10 <sup>3</sup> c ells/MCL)	NEU (%)	NI (%)	NA(%)
BN	5.22	30.56	1611	4.42	26.36	1187	26.32
TUM	5.10	31.18	1590	4.25	28.21	1199	24.59
GAR	4.27	33.60	1435	3.77	31.08	1172	18.33
NEE	4.02	34.92	1404	3.62	32.46	1175	16.31
VC	3.71	34.71	1288	3.31	32.31	1069	17.00
CON	3.38	35.50	1199	3.08	33.11	1020	14.93

BN = Baphia nitida; TUM = TumeriViC; GAR = GarliViC; NEE = NeemViC; VC = Vitamin C; CON = Control

## DISCUSSION

The increase in the lymphocytes and red blood cells (RBCs) associated with the present study supported the findings of Yapo *et al.*, (2011), Sumalatha *et al.*, (2012) Anarthe *et al.*, (2014) and Obi *et al.*, (2019) but disagree with the findings of Johnson *et al.*, (2017). Variations in the values of immune cells/blood cells observed in the present study could be attributed to the variation in the ability of the plant supplements to argument the hematopoietic processes in the cells of the experimented chicks. An increase in the hematological indices was observed in broiler chicks administered plant extracts as documented by Mwale *et al.* (2013). Several researchers had reported similar effect on broiler chicks (Bonsu *et al.*, 2012; Ewuola and Egbunike, 2008; Ganesan and Bhatt, 2008; Huff *et al.*, 2008; Mwale and Masika, 2009; Mwale and Masika, 2011; Mwale and Masika, 2012; Mwale *et al.*, 2013). The increase in the hematological indices could be attributed to high anti-oxidative potentials of the plant extracts as reported by Mwale *et al.* (2013). The increase observed in the hematological indices showed that the plant extracts are capable of promoting and stimulating the activities of immune system as documented by Bonsu *et al.* (2012). The increase could also be

attributed to the ability of the plant extract to inhibit process of oxidative break down of erythrocyte as reported by Ewuola and Egbunike (2008). The presence of phytochemicals such as flavonoids, tannins, and terpenes in the plant extracts could be responsible for the increase in hematological indices as documented by Mwale and Masika (2008). The ability of the plant extracts to stimulate stem cells in the bone marrow could be responsible for the increase in the hematological indices in the broiler chicks as reported by Mwale and Masika (2011). Mwale and Masika (2011) also attributed the increase to the action of erythropoietin, which is a glycoprotein hormone.

Murithi *et al.* (2015) documented an increase in neutrophil count in broiler chicks administered plant extracts. This observation was supported by findings of several researchers (Sumit *et al.*, 2014; Mansi and Lahham, 2008; Kuppast *et al.*, 2009). The increase in the neutrophil count could be due to the fact that the plant extracts contain agents that stimulate the bone marrow to produce neutrophils and release them into the blood (Mansi and Lahham, 2008). The increase could also be attributed to high anti-oxidative activity of the plant extracts as documented by Sumit *et al.*

(2014). ). The increase in the total neutrophil count could be attributed to the ability of the plant extract to enhance formation processes (Esenowo *et al.*, 2010). The increase was also ascribed to the activation of the immune system, a normal cell-mediated immune response by the plant extracts (Okon *et al.*, 2013). The increase in the total neutrophil count could be attributed to an increase in the dosage of the plant extract as reported by Wambi *et al.* (2008).

**CONCLUSION:** The study has shown that the plant supplements exhibited pronounced increase in lymphocytes, neutrophils, WBC and absolute lymphocytes of which BN was most effective, and these proved that the plant supplements had immune support potential.

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