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UPSHOT OF PLANT SUPPLEMENTS ON BODY WEIGHTS AND HUMORAL ACTIVITIES OF BROILER CHICKS

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ABSTRACT

Studies have shown that daily intake of some botanical products supplemented with vitamin C supersize the activities of immune cells and organs in the body. This study was carried out to evaluate the upshot of plant supplements on body weights and humoral activities of broiler chicks. Equal concentration (100 mg/mL) of extracts from *Azadirachta indica* leaves (Neem plant), *Curcuma longa* roots (turmeric) and *Allium sativum* rhizomes (garlic) were mixed with equal concentration (100 mg/mL) of vitamin C to prepare NeemViC (NE), TumeriViC (TU), and GarliViC (GA), respectively. A 0.5 mL/100 g of *Baphia nitida* leaf extract (BN), GA, NE, and TU each was orally administered to 3 weeks old broiler chicks, and body weights assessed and the humoral activity from the blood samples drawn from the chicks after 11 days. There was progressive increase in the body weights of the experimented broiler chicks in every 2 days intervals, but this was statistically non-significant ($P > 0.05$) as compared to the control group. There was significant ($P<0.05$) increase in anibody titre values of blood samples drawn from the experimented chicks, of which those chicks administered TU recorded the highest titre value (426.67 HA unit/mL) as compared to the control (106.67 HA unit/L). Therefore, the plant supplements exhibited pronounced increase in the body weights and humoral activities, and TU was most effective.

Keywords: Immune cells, Body weights, Extracts, Organs

INTRODUCTION

Background of study

The immune system contributes significantly to human health. Malfunctional immune system results in various diseases like arthritis, ulcerative colitis, asthma, allergy, cancer and infectious diseases [Abid *et al.,* 2012]. Such diseases, caused by modulation of immune responses, have been of interest for many years. Substances which can affect the immune system are known as immunomodulators. Immunomodulators can be immunostimulant or immunosuppressant [Rasheed *et al*., 2016]. Recently, a number of synthetic as well as natural immunomodulatory agents have been introduced in order to modulate non-specific andspecific immune responses. Currently available chemotherapeutic agents mainly have immunosuppressive activity and most of them are cytotoxic and exert a variety of side effects.Therefore, the medicinal plants and their isolated bioactive components with immunomodulatory potential are gaining importance to discover alternative immunomodulatory agents. Several medicinal plants with established immunomodulatory potential have been documented by several researchers (Abid *et al.,* 2012; Rasheed *et al*., 2016]. Viscum album, Panax ginseng and Tinospora cordifolia, have been shown to alter the immune function . The usage of medicinal plants with immunostimulatory effects in patients, reported less toxicity and side effects [Emadi *et al*., 2009]. Researchers around the world are focusing to explore medicinal plant and plant derived substances which can alter certain immune responses. Thus, these natural substances can replace conventional chemotherapies for modulation of immune response, particularly in impaired host immune response. The current study was aimed to explore the immunomodulatory activities of methanol extracts of *Mangifera indica* leaves and *Curcuma domestica* rhizomes on innate and adaptive immune response in male albino ICR mice [Abid *et al.,* 2012].

Understanding the immune system may help in improving immunization protocols inhuman, as well as, animals to develop vaccines, which play an important role in the preventive medicine and provide a rational basis for devising new therapeutic strategies for immune mediated diseases. In this regard, immunomodulators are plants and plant products, or biological materials that mediate the effectors mechanisms of the immune system through immune stimulation to a given antigen or potentiate the effectiveness of

a vaccine (Akihisa *et al*., 2009). Scientists have begun to adopt vaccine strategies that are based on the maximization of antigen presentation for major histocompatibility complex (MHC) classI or class II molecules due to the importance of these molecules in immune response, especially those materials that act as immunomodulators .Materials of fungal and/or plant origins have been the interest of different investigators around the globe with their aims to establish the immunomodulator potentials of these materials. Some risks associated with attenuated or killed whole-organism vaccines can be avoided with vaccines that consist of specific purified macromolecules derived from pathogens or in combination with plant materials (Akihisa *et al*., 2011). The plant extracts, derivatives or their products, have also been the interest of investigators as immunomodulators to overcome the disadvantage of biological and chemical immunomodulators. One of these plants is

Azadirachta indica, which is more popular with the name neem, and has the advantage to be a medicinal plant with a wide range of applications in folkloric medicine.

Furthermore, recent investigations demonstrated several biological and pharmaceutical potentials; for instance, anti-viral, anti-bacterial, anti-parasitic, anti-cancer and immune stimulant properties of the Neem (Al-Quraishy *et al*., 2012). This research is aimed at evaluating the upshot of plant supplements on body weights and humoral activities 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 ± 2^0C 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 α-naphthol was add ed 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 prepare 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_2O_2) 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 37^0C 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 miniprepTM 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 ($MgCl_2$) 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 H5N1virus 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 *Azadirchta 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 body weights and blood absolute lymphocytes were assessed from the blood samples drawn from the chicks after 11 days.

Body weights: The body weights of the experimented rats were checked and recorded weekly using electronic weighing balance (LXD200) and recorded as described in the work published by Nwobodo *et al*. (2018),

Ejike *et al*. (2017), Nwobodo *et al*. (2018) and Ekesiobi *et al*. (2025).

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Humoral activity of the Plant Supplements: This was carried out using the modified methods described and published by Anartle et al. (2014), Ntambi et al. (2015), Iheukwumere *et al*. (2022a) and Iheukwumere and Iheukwumere (2022a). On 7th day, the broiler chicks were challenged with the prepared antigen, blood samples were drawn from tails and ears of the broiler chicks in every 3 days intervals. The blood samples were centrifuged for 2000 xg for 5 min, and the sera were collected. Two -fold serial dilution was carried out on each of the serum (ie 50µl of serum was added in each microtiter well dilution in order to get up to the 24^{th} well. Then 50µl of 1% SRBC prepared using normal saline was added into each well, and the plates were incubated at 37^{o}C for 1h. The reciprocal of the highest dilution that showed visible agglutination was considered as the hemagglutination antibody titer (HA units /NL).

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 revealed pronounced increase in body weights in every two days interval as shown in Table 1. The maximum weight increase was seen after 2 days, and slight retardation was observed after 4, 6, 8, and 10 days, respectively. It was also observed that the increase in the weights of the experimented broiler chicks administered the plant supplements were higher than that of the normal administered garliViC that showed slight decrease in weight when compared to the control group.

The study revealed pronounced increase in antibody titer as shown in Table 2. The antibody titer increased significantly ($P<0.05$) above the control group, and also increased in every 3 days interval. TumeriViC recorded the highest antibody titer, followed by GarliViC, Baphia nitida, NeemViC, and Vitamin C recorded the least titer value. It was also observed that the maximum antibody production was observed after 6 days before slight retardation as shown in Table 2.

Table1: Body weight of the experimented broiler chicks

Day	Mean weight (g)					
	GarliViC	NeemViC	Baphia nitida	TumeriViC	Vit C	Con
0	214.86±4.12	226.13±3.87	228.11±5.12	222.03±5.22	219.16±4.11	223.41±3.22
2	237.14±2.92	268.22±4.24	291.11±3.16	282.17±2.76	261.46±2.19	252.86±2.12
4	271.11±3.36	318.22±3.72	346.23±2.92	332.86±4.22	301.24±2.46	302.92±2.51
6	322.86±5.02	331.76±2.19	383.19±2.41	376.12±2.62	341.92±3.11	325.56±3.06
8	361.22±2.71	376.18±3.11	413.92±4.12	407.22±3.22	371.01±2.27	365.22±2.51
10	383.46±3.13	396.31±2.81	431.86±2.61	423.11±3.31	394.12±4.03	386.14±2.12

Table2: Effect of the plant supplement on antibody secretion in the broiler chicks

Sample	Mean Antibody (HA Unit/ mL)			
	Day 0	Day 3	Day 6	Day 9
Baphia nitida	16.00	42.67	213.33	256
Tumeric	16.00	64.00	256.00	426.67
GarliViC	16.00	42.67	213.33	341.33
NeemViC	8.00	32.00	170.67	213.33
VitaminC	8.00	26.67	106.67	128.00
Control	8.00	21.33	85.30	106.67

DISCUSSION

Abo Omar *et al*. (2016) reported an increase in body weight of broiler chick administered medicinal plant extract, which disagrees with the finding of Yazdy *et al*. (2014) who recorded zero effect of plant extract on the growth of broiler chick. Several researchers documented a significant improvement on the weight of broiler chicks (Toghyani *et al*.,2010; Najafi and Turki, 2010; Elbushra, 2012; Daramola, 2019). The increase in the body weight was attributed to increased secretion of digestive enzymes which digest more body building nutrients such as aminoacid (Abedin *et al*., 2019). Toghyani et al. (2010) attributed the increase in the weight of broiler chicks to the presence of essential fatty acid. The increase in the weight of chick was attributed to improvement of anti-oxidative capacity as reported by Daramola (2019).

The significant increase in antibody titer is an indication that antibody production to T-dependent antigen SRBC requires cooperation of T and B lymphocytes and macrophages and these evolved immunostimulation through humoral immunity. Similar deduction was made by Anarthe *et al*., (2014). In the present study, the plant supplements were able to influence the roles of immunoglobulins in order to activate pre B cells and dendritic cells, and these result produce antibodies. Thus the titer values against SRBC increases. Similar observation was reported by Anarthe *et al*., (2014). Bittencourt *et al*. (2007) documented a substantial increase in antibody production in broiler chicks administered plant extracts. Similar findings were reported by several researchers (Bragagnolo *et al*., 2007; Silveira *et al.,* 2009; Orsatti *et al*., 2010a; Orsatti *et al*., 2010b). The increase in the number of antibody could be attributed to the presence of phytochemicals such as flavonoids, saponins, and organic acids in the plant extracts which stimulate antibody-producing plasma cells as reported by (Silveira *et al.,* 2009). Orsatti *et al*., 2010a) reported that proteins found in the plant extracts could be responsible for high antibody production. Orsatti *et al*. (2010b) reported that the increase could be attributed to optimum dosage of the medicinal plant extracts. Also, the mechanism of plasma cell proliferation is stimulated by the medicinal plant extracted as documented by Bragagnolo *et al*. (2007). Orsatti et al. (2010b) also reported that the increase could be attributed to high antioxidant potentials of the plant extracts.

CONCLUSION: The study has shown that the plant supplements exhibited pronounced increase in body weights and humoral activities of which TumeriVic was most effective, and these proved that the plant supplements had immune support potential.

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