Justicia Secunda: a Poultry Phytogenic Growth Promoter

Augustine Okpani Oko (augustine.oko@ebsu.edu.ng)
Ebonyi State University https://orcid.org/0000-0001-7450-8120
Emmanuel Toochukwu Ekuma
Ebonyi State University
Stanley Chijioke Eluu
Ebonyi State University

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Abstract

Aqueous and ethanol leaf-extracts of *Justicia secunda*, were investigated for possible application as poultry growth promoters. Vitamins, minerals and phytochemical compositions of the leaves were investigated using standard methods. Seventy-two broilers were grouped into nine with eight birds per treatment through random selection and bred for period of eight weeks. Growth and meat quality evaluations, biochemical and hematological assessment of the blood were done using standard methods. Results revealed that the plant contained water-soluble vitamins, fat-soluble vitamins, macro-elements, trace-elements, alkaloids and polyphenols necessary for improving poultry performances. It was observed that the extracts improved meat quality of the broilers more than control groups, as the drip and cooking losses of the broilers were lower. Also, there were improved blood proteins (total protein, albumin, globulin) and hematological indices of the broilers. Consequently, leaf-extracts of *J. secunda* could serve as replacements for chemical growth promoters with aqueous extracts as the better choice having produced better effects than ethanol extracts.

Introduction

The primary purpose of poultry production is to attain optimum production at minimum possible costs in meeting the increasing demand for animal protein and providing safe food for human beings that is free from chemical additives. In Africa, especially Nigeria, there has been an unparalleled growth in poultry production, which has been largely secluded to the large- and small-scale organized poultry industries, which has been primarily achieved through the exploitation of various modern growth promoting strategies (Kataria et al., 2005).

Basically, poultry producers use growth promoters (such as polarmix, anidone multivitamin, albiovit, B-vit extra, VITA nutrition etc.) containing vitamins, minerals and sometimes, antibiotics at sub-therapeutic levels, to improve growth and general health of the birds. However, the prolonged use of these growth promoters, especially those containing antibiotics, has been reported to exhibit detrimental reproductive effects on poultry. They induce livestock antibiotic resistance, and lead to the accumulation of chemical residues in the bodies of the birds, which is potentially passed to humans, (Achilonu et al., 2018). In the same vein, it has been reported that chemical growth promoters administered to birds are excreted into the environment through urine and feces (Carvalho and Santos, 2016), predisposing sewage disposal systems as important routes whereby toxic substances can enter the environment (Ronquillo and Hernadez, 2016), thereby contaminating the confined aquatic ecosystems such as ponds and lakes, as well as soil close to the poultry sites.

Consequently, poultry production sectors are facing the problems of increase demand for a better and more effective growth promoters (minerals and vitamin supplements), to boost growth, improve the general health of the birds, without competing with human food supply chain (Moreki and Gabanakgosi, 2014). This situation has necessitated the search for cheaper and locally available substitutes that can compete favorably the available chemical growth promoters.

In this regard, poultry scientists are now trying out other possible alternatives such as the use of probiotics, prebiotics and organic acids, which may play a role in possible therapies to improve the health as well as the general performance of the birds, and safe for human society (Aljumaah et al., 2020; Taer et al., 2020). Phytogenic growth promoters are the most widely sort replacement for chemical growth promoters in poultry production due to their several medicinal properties with little or no side effects (Taer et al., 2020; Kikusato, 2021, Krauze et al., 2021).

Phytogenic growth promoters, otherwise known as phytobiotics, are broad subset plants-derived bioactive compounds incorporated into the diets to improve livestock productivity through amelioration of feed properties, improvement of nutrient digestibility, absorption and elimination of pathogens in the gut (Kikusato, 2021). These substances are beneficial in poultry nutrition because of their active pharmacological compounds that stimulate appetite and feed intake, improve endogenous digestive secretion, activate immune responses and improve the quality of products derived from poultry (Taer et al., 2020). *Justicia secunda* (Vahl), which have been reported to contain some active pharmacological compounds, may fall under this category.

J. secunda, a creeping perennial herb, native of Africa, Madagascar, and Asia, belongs to the family of Acanthaceae and is cultivated majorly as a garden ornamental plant (Mea *et al*., 2017). In Congo and South Cote-d'Ivoire, the Jehovah's Witness believers are known for their refusal of blood transfusion, hence, they take leaf decoction of *J. secunda* for the management of anemia, while the boiled water extract of the aerial parts of *J. secunda* is used in Venezuela as antipyretic and in the treatment of chicken pox (Abo *et al*., 2016). Phytochemical studies of the leaves of the plant have been known to possess some phytogenic compounds such as tannins, flavonoids, alkaloids, phenols, minerals and vitamins (Mea *et al*., 2017) which supports the findings of some pharmacological properties such as anti-viral, anti-tumoral, anti-microbial, anti-sickling; anti-inflammatory and anti-platelet-aggregation possessed by the plant (Osioma and Hamilton-Amachree, 2017).

Materials And Methods

Collection, identification and preparation of plant samples

The leaves of *J. secunda* were collected from plants growing in Amaizu community in Afikpo North Local Government Area of Ebonyi State during the raining season. The fresh and clean leaves were air dried under a shed until they were crispy to touch, while retaining their colorations. The leaves were powdered using corona manual grinder. Aqueous and ethanol extracts were prepared. The ethanol extraction was done using the Soxhlet apparatus to obtain about 1.5 kg of extract (Patel *et al.,* 2019).

Vitamin analysis

A calibration curve for each of vitamin A, E, C and D, at different concentrations were prepared and absorbance obtained using a spectrometer (APEL 3000 uv). Vitamins were determined using standard methods by Kirk and Sawyer (1991).

Minerals analyses

Elemental analysis was conducted using Agilent FS240AA Atomic Absorption Spectrometer according to the method of APHA 1995 (American Public Health Association). The sample was thoroughly mixed by shaking, and 100 ml of it was transferred into a glass beaker of 250 ml volume, to which 5 ml of concentrated nitric acid was added and heated to boil till the volume was reduced to about 15 – 20 ml, by adding concentrated nitric acid in increments of 5 ml till all the residue was completely dissolved. The mixture was cooled and made up to 100 ml using metal free distilled water. The sample was aspirated into the oxidizing air-acetylene flame. When the aqueous sample was aspirated, the sensitivity for 1 % absorption was observed (APHA, 1995).

Phytochemical screening

One gram (1 g) of sample was weighed and transferred to a test tube. Fifteen milliliters (15 ml) of ethanol with 10 ml of 50 %m/v potassium hydroxide (Hawkins, USA) were added in the test tube. The mixture was allowed to react in a water bath at 60 ^{o}C for 1 h. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel containing 20 ml of ethanol, 10 ml of cold water, 10 ml of hot water and 3 ml of hexane (Yufeng, Japan). The mixture was washed three times with 10 ml of 10 %v/v ethanol solution. The solution was dried with anhydrous sodium sulfate (Hawkins, USA) and the solvent was evaporated.

The sample was solubilized in 1000 µl of pyridine of which 200 µl was transferred to a vial for analysis. The analysis was performed in a Gas chromatography (BUCK M910) equipped with a flame ionization detector (GC-FID). A RESTEK 15 m MXT-1 column (15 m x 250 m x 0.15 µm) was used. The injector temperature was 280 ^{o}C and a linear velocity of 30 cm/s. Helium 5.0 pa.s was the carrier gas with a flow rate of 40 ml/min. The oven operated initially at 200 ^{o}C. It was heated to 330 ^{o}C at a rate of 3 ^{o}C/min and was kept at this temperature for 5 min. The detector operated at a temperature of 320 ^{o}C. The concentrations of the phytochemicals were calculated as the ratio between the area and mass of internal standard and the area of the identified phytochemicals. These concentrations were expressed in µg/g.

Experimental animals

A total of seventy-two (72) day old chicks weighing between 48 g to 50 g were purchased from Rosambro Broilers in Abakaliki, Ebonyi State. The chicks were housed in a battery system, randomly divided into nine (9) groups of eight (8) birds per treatment and left in a good environmental condition. Feed and water were provided *ad libitum* and all required management practices applied as at when due (Sarker *et al.,* 2016).

Acute toxicity test

This study was carried out using the up and down method of acute toxicity (OECD, 2008). Nine (9) broilers were randomly selected, weighed and divided into 3 groups of A, B and C. Each group was made

up of 3 broilers. Group A received 2.0 g/l aqueous extract of *J. secunda*, group B received 2.0 g/l ethanol extract of *J. secunda*, while group C was given equal volume of distilled water. These were administered orally through mixing with drinking water. The broilers were observed for 48 h for signs of toxicity and mortality (OECD, 2008).

Ethics and animal welfare

Animal care and procedures were performed by following the guidelines of good experimental practices. The Ebonyi State University Ethical Committee granted permission for this research to be conducted.

Experimental design

Complete randomized design was used in this analysis. Nine (9) treatment groups were created and designated as T_1; T_2; T_3; T_4; T_5; T_6; T_7; T_8; and T_9. The seventy-two (72) experimental broilers were assigned to the treatment groups and each group consisted of eight chicks as illustrated below. Each treatment group received normal feed and water containing different concentrations of the leaf-extracts.

Group 1 (T_1) was given 0.25 g/l ethanol extract of *J. secunda*,

Group 2 (T_2) was given 0.50 g/l ethanol extract of *J. secunda*,

Group 3 (T_3) was given 0.75 g/l ethanol extract of *J. secunda,*

Group 4 (T_4) was given 0.25 g/l aqueous extract of *J. secunda,*

Group 5 (T_5) was given 0.50 g/l aqueous extract of *J. secunda,*

Group 6 (T_6) was given 0.75 g/l aqueous extract of *J. secunda,*

Group 7 (T_7) was given 0.50 g/l Albiovit (a commercial growth promoter)

Group 8 (T_8) was given 0.50 g/l B-vit extra (a commercial growth promoter)

Group 9 (T_9) was given normal diet without any growth promoter

Groups 1 – 6 were the study groups. Group 7 and Group 8 served as the positive control groups, while Group 9 served as negative control group. The growth promoters were administered to the chickens through adding them in their drinking water for a complete 8 weeks period (Sarker *et al.,* 2016).

Carcass yield and meat quality evaluation

The chickens were fed normal diet (feed + water) plus experimental growth promoters in the morning (08.00 h) *ad libitum* in separate feeders, so the chicks determined their intake of the feed. The diets were

offered daily and water was also provided *ad libitum* in plastic containers. All birds were weighed at the start of the experiment before allocating them to the treatments and after 56th day (final day) of the experiment. Also, consumed diets were recorded for calculation of weight gain, feed conversion ratio, and specific growth rate according to methods described by Sarker *et al.* (2016).

At the end of the experiment, five broilers per treatment (45 broilers in all) were randomly selected, starved of feed for 10 h and sacrificed by cutting the jugular vein to allow proper bleeding. Determination of blood weight was by the difference between slaughter weight and hot carcass weight. The weights were measured using Camry manual weighing scale. The broilers were defeathered by scalding in hot water at 60 ^{o}C and eviscerated to evaluate their carcasses.

The breast meats of the broilers were removed, weighed, and suspended in the deep freezer for 48 h. After 48 h freezing period, the meat was thawed, weighed, and cooked for 30 min after it started to boil. After the cooking period, the meat was allowed to cool and the final weight recorded. The drip loss was measured as the weight loss during suspension of the breast meat in an airtight container over 48 h at 4 ^{o}C. Drip loss was expressed as a percentage relative to the initial weight according to the standard method of Honikel (1987). The cooking loss was calculated by the method of Jama *et al.* (2008) and expressed as:

$$\text{Cooking loss} = \frac{(\text{weight of meat after thawing} - \text{weight of cooked meat}) \times 100}{\text{Weight of meat after thawing}}$$

Biochemical analysis of blood

At the end of the feeding period (after 56 days from the beginning of the experiment), the birds were starved of feed for 10 h before blood samples were collected from five chickens per treatment for biochemical and hematological analyses. A 2 ml of blood samples was collected from the femoral vein into labeled sterile sample bottles containing no anticoagulants, using a sterilized disposable syringe and needle. This was used to determine the biochemical parameters. Prior to bleeding, a cotton swab soaked in 70 % ethanol was used to dilate the vein and to prevent infection. The blood samples without anticoagulants were centrifuged at 500 rpm (revolution per minute) for 3 min in a micro-centrifuge to obtain serum that was free from cell debris.

The serum obtained was analyzed colorimetrically for total protein (TP) by the Biuret method with kits as described by Dawnay *et al.* (1991). Two grams (2 g) of sample was treated with an equal volume of 1 % of sodium hydroxide, followed by a few drops of aqueous copper (II) sulphate. A blue color was formed. The intensity of the blue color formed was proportional to the protein concentration in the plasma or serum. The mixture was incubated at room temperature for 20 min and read at a wavelength of 560 nm using a spectrophotometer.

Albumin (Ab) concentration was determined by the Bromo cresol Green (BCG) method (Peters *et al.*, 1982). The sample was diluted with 0.85 % sodium chloride and 2 drops of BCG was added which resulted in a color shift from yellow to green. The concentration of Ab was directly proportional to the intensity of the green color formed. The mixture was incubated at room temperature for 5 min and read with a spectrophotometer at a wavelength of 620 nm.

Globulin (Gb) concentration was calculated as the difference between total protein and albumin concentrations.

Hematological analysis of the blood

An initial 2 ml blood was collected from the drumstick veins of the birds into labeled sterile universal bottles containing Ethylene-Diamine-Tetra-Acetic Acid (EDTA) as anticoagulant. This was used to determine the hematological components within an hour of sample collection. The blood containing EDTA was mixed very well by inverting several times. Then the capillary tube was filled up to two-third the entire length, with one end of it sealed with plasticine. The tube was placed in a hematocrit machine and centrifuged for 5 min at 3000 rpm. PCV was calculated as follow:

$$PCV = \frac{L_2 \text{ x } 100}{L_1}$$

Where L_1 is the upper layer of the capillary tube after centrifugation

L_2 is the lower layer of the capillary tube after centrifugation

Hemoglobin was determined using the sodium lauryl sulphate (SLS) method according to Oshiro *et al.* (1982). A 25 µl of blood was mixed with 5 ml of a 2.08 mmol/l solution of SLS (buffered to pH of 7.2). The mixture was read with a spectrometer at 539 nm.

The WBC count was determined using Battlemen method of counting WBC. A dilution of the EDTA containing blood was carried out. That is, 1 drop of the sample in 19 drops of Turk's solution. The dilution was allowed to stand for about 5 mins for the red blood cells (RBCs) to lyse. The Neubauer counting chamber was charged and filled with the dilution. The chamber was allowed to stand for 5 min for the WBCs to distribute evenly. A microscope, focused with x10 and viewed with x40 objective lenses was used to count the WBCs. The counting was done on four large squares. The WBC was calculated as follow:

$$WBC = \frac{N \text{ x } Df}{V}$$

Where N is number of WBCs counted

Df is dilution factor

V is volume of 4 large squares which is given as 4 x 0.1 mm^3

The neutrophils, lymphocytes and eosinophils were determined using methods described by Nakul *et al.* (2003). On a clean grease free microscopic slide, 0.6 µl of the EDTA containing blood was added. Using a suitable spreader (a slide) 1 cell thick film was made and allowed to air dry on a safe non-contaminating area and then stained with Leishman for about 5 – 10 mins and then rinsed and allowed to air dry again. It was then taken to the microscope for the differential WBC analysis (Oshiro *et al.,* 1982; Nakul *et al.,* 2003).

Statistical analysis

Analysis of variance (ANOVA) for Completely Randomized Design (CRD) was carried out using Statistical Package for Social Sciences (SPSS). Values were presented as mean ± standard deviation (mean ± SEM). Differences between means were separated using the Least Significant Difference (LSD), with a p-value of less than 0.05 ($p < 0.05$) as the level of significance (Seaman *et al.,* 1991). Principal component analysis was performed using R software.

Results

Vitamin compositions of the plants

The vitamins analysis of the extracts showed that the plant contained the following vitamins: vitamin A, vitamin E, vitamin C, vitamin D, vitamin B_1, vitamin B_2, vitamin B_3, vitamin B_6 and vitamin B_{12} in different proportions, as presented in Table 1. There were significant differences ($p < 0.05$) observed across the extracts for each of vitamin E, vitamin C, vitamin D and vitamin B_3. The vitamin A content of the aqueous extract was not significantly different ($p > 0.05$) from that of the ethanol extract.

Again, no significant differences ($p > 0.05$) were noticed in the extracts for vitamin B_1, and vitamin B_6 respectively. Furthermore, it was observed that the vitamin C content of the aqueous was significantly higher ($p < 0.05$) than that of the ethanol extract. On the other hand, the ethanol extract was high in vitamin A, vitamin E and vitamin D compared to the levels of these vitamins in the aqueous extract.

Table 1

Vitamin composition of leaf-extracts of *J. secunda*

	J. secunda (Aqueous)	*J. secunda* (Ethanol)		
			SEM	P
Vitamin A (µg/g)	72.756[a]	73.644[a]	0.2780	0.013
Vitamin E (µg/g)	29.725[b]	68.206[a]	0.3495	0.000
Vitamin C (µg/g)	88.115[a]	87.096[b]	0.2229	0.002
Vitamin D (µg/g)	24.026[b]	38.362[a]	0.2831	0.000
Vitamin B_1 (mg/g)	0.158[a]	0.168[a]	0.0397	0.807
Vitamin B_2 (mg/g)	0.1587[b]	0.213[a]	0.0113	0.001
Vitamin B_3 (mg/g)	3.5868[b]	4.392[a]	0.2111	0.005
Vitamin B_6 (mg/g)	0.625 [a]	0.595[a]	0.0979	0.767
Vitamin B_{12} (mg/g)	0.163[a]	0.047[b]	0.0356	0.012

Different letters indicate significant difference across the row ($p < 0.05$), where a > b

Mineral compositions of the plant

The results of the mineral compositions of the extracts are shown in Table 2. The study showed that calcium, magnesium, potassium, sodium, phosphorus, iron, arsenic, zinc, copper, selenium and molybdenum were present in both the ethanol and aqueous extracts of the plant. Manganese and cobalt were also present in aqueous extract but not in the ethanol extract of the plant. Significant differences ($p < 0.05$) were observed across the leaf-extracts for magnesium, sodium, potassium, calcium, iron, molybdenium, arsenic, selenium, cobalt, manganese and copper contents of the plant.

Table 2

Mineral composition of the leaf-extracts of *J. secunda*

Parameters	*J. secunda* (aqueous)	*J. secunda* (ethanol)		
			SEM	P
Copper (mg/g)	0.135^{b}	0.147^{a}	0.0029	0.004
Magnesium (mg/g)	5.337^{b}	5.612^{a}	0.0318	0.000
Sodium (mg/g)	2.289^{b}	3.920^{a}	0.0047	0.000
Potassium (mg/g)	8.888^{b}	9.071^{a}	0.0314	0.000
Molybdenum (mg/g)	0.043^{a}	0.028^{b}	0.0026	0.000
Calcium (mg/g)	0.473^{b}	8.084^{a}	0.0242	0.000
Iron (mg/g)	5.183^{a}	5.002^{b}	0.0411	0.002
Selenium (mg/g)	0.642^{a}	0.488^{b}	0.0090	0.000
Phosphorus (mg/g)	0.001^{b}	0.006^{a}	0.0017	0.000
Zinc (mg/g)	1.989^{a}	1.442^{b}	0.0407	0.000
Cobalt (mg/g)	0.117^{a}	NIL	0.0017	0.000
Manganese (mg/g)	0.206^{a}	NIL	0.0030	0.000
Nickel (mg/g)	0.007^{a}	0.001^{b}	0.0010	0.001
Arsenic (mg/g)	0.0833^{a}	0.062^{b}	0.0016	0.000

Different letters indicate significant difference across the row ($p < 0.05$), where a>b.

Phytochemical compositions of the plants

The results of the phytochemical contents of the extracts are shown in Table 3. It was observed that flavanones, flavan-3-ol, flavones, tannins, phenols, oxalate, sapogenin, kaempferol, steroids, epicatechin, epihendrine, naringenin, and lunamarin were contained in the aqueous and ethanol extracts of the plant in different proportions.

The results further showed that phytate was contained in aqueous extract but not in the ethanol extract of *J. secunda.* It was observed that these phytochemicals: flavones, phenols, sapogenin, tannins, anthocyanin, steroids, and quinine were not significantly different ($p > 0.05$) across the extracts. Again, the result showed that the aqueous leaf-extract of *J. secunda* contained significantly higher ($p < 0.05$) values of flavan-3-ol, flavonones, phenols, sapogenin, phytate and steroids compared to the ethanol extracts of the plant

Table 3

Phytochemical composition of the leaf-extracts of *J. secunda*

	J. secunda (Aqueous)	*J. secunda* (Ethanol)		
			SEM	P
Flavan-3-ol (µg/ml)	20.796[a]	13.879[b]	0.1867	0.000
Flavone (µg/ml)	5.97[b]	17.880[a]	0.2108	0.000
Flavonones (µg/ml)	12.672[a]	11.293[b]	0.3090	0.002
Phenol (µg/ml)	18.763[a]	9.359[b]	0.1907	0.000
Oxalate (µg/ml)	6.655[b]	7.728[a]	0.2955	0.007
Sapogenin (µg/ml)	24.195[a]	11.407[b]	0.3176	0.000
Phytate (µg/ml)	11.234[a]	NIL	0.2506	0.000
Tannins (µg/ml)	13.665[b]	34.041[a]	0.3196	0.000
Kaempferol (µg/ml)	17.0724[b]	28.596[a]	0.2621	0.000
Proanthocyanin (µg/ml)	7.963[a]	7.412[a]	0.1671	0.070
Anthocyanin (µg/ml)	NIL	17.605[a]	0.0939	0.000
Steroids (µg/ml)	8.815[a]	4.259[b]	0.2587	0.000
Epicatechin (µg/ml)	3.784 [a]	2.832[b]	0.4013	0.045
Catechin (µg/ml)	25.538[a]	NIL	0.2415	0.000
Epihendrine (µg/ml)	6.629[b]	9.393[a]	0.3990	0.000
Ribalinidine (µg/ml)	15.748[b]	18.990[a]	0.2832	0.000
Naringenin (µg/ml)	20.521[a]	6.802[b]	0.2124	0.000
Naringin (µg/ml)	4.160[a]	NIL	0.2147	0.000
Rutin (µg/ml)	11.492[b]	14.434[a]	0.3783	0.000
Lunamarin (µg/ml)	10.275[b]	12.223[a]	0.2092	0.000
Quinine (µg/ml)	12.230[a]	4.663[b]	0.1878	0.000
Spartein (µg/ml)	NIL	3.751[a]	0.2387	0.000

Different letters indicate significant difference across the row ($p < 0.05$), where a > b > c > d

Acute toxicity test

Oral administration of 2.0 g/l of the leaf-extracts and equal volume of distilled water produced no death or any sign of toxicity after 48 h.

Carcass yield and meat quality

The chickens had weight gain values that were dependent on the dose of the extracts administered. The weight gain of the groups given aqueous extracts increased with increasing concentrations, whereas, that of the groups fed ethanol extracts increased with decreasing concentrations as shown in Figure 1. The group fed 0.75 g/l aqueous of *J. secunda* leaf-extract had the highest weight gain value of 3.0177 ± 0.1155. The least weight gain was observed at the negative control group which had a value of 2.0847 ± 0.1158 that was significantly lower ($p < 0.05$) than the weight gain values obtained from other groups.

It was also observed that the groups that were given 0.75 g/l aqueous extract had weight gain values that were not significantly different ($p > 0.05$) from the groups fed 0.50 g/l aqueous extract. It was further observed that the weight gain values of the groups given 0.75 g/l and 0.50 g/l aqueous extract were significantly higher ($p < 0.05$) than the weight gain values of other groups. The weight gain values of the groups fed the extracts were not significantly different from the values of the groups fed commercial growth promoters.

The specific growth rate values obtained, were dependent on the dose of the extracts administered. The groups given aqueous extracts had specific growth rate values that increased with increasing concentrations, whereas, the groups fed ethanol extracts gave specific growth rate values that increased with decreasing concentrations. The group fed 0.75 g/l aqueous leaf-extract of *J. secunda* had the highest specific growth rate value of 7.39 ± 0.067. The least SGR was observed at the negative control group which had a value of 6.75 ± 0.110 that was significantly lower ($p < 0.05$) than the specific growth rate obtained from other groups. Also, it was observed that the groups that were given 0.75 g/l and 0.50 g/l aqueous extracts had SGR that were significantly higher ($p < 0.05$) than the groups fed with ethanol extracts. The specific growth rate values of the groups fed aqueous extracts were not significantly different from the SGR of the groups fed commercial growth promoters.

The highest drip loss observed after the experiment was 5.0 ± 0.8824 % for the negative control group. This value was significantly higher ($p < 0.05$) than the values obtained for the other groups. No drip loss was recorded for the groups fed aqueous leaf-extracts and the groups fed commercial growth promoters. Again, groups fed ethanol extracts had drip losses that were higher than those observed for the groups fed aqueous extracts. Nevertheless, no significant differences ($p > 0.05$) were observed in the drip losses of all the test groups.

The cooking losses observed from the study were dependent on the dose of the extracts administered. The groups given aqueous leaf-extracts had cooking loss values that increased with decreasing concentrations, whereas, the groups fed ethanol extracts gave cooking loss values that increased with increasing concentrations. It was observed from the study that none of the groups had up to 50 % cooking loss. The negative control group had the highest cooking loss value of 45.0110 ± 1.1492 %

which was significantly higher than the cooking losses obtained in other groups. There were no significant differences ($p > 0.05$) observed in the cooking loss values of the test groups.

Blood protein

The total protein values of the groups increased with reducing concentration of the leaf-extracts as presented in Figure 2. The least TP value was observed at the negative control group with a value of 19.32 ± 0.877 g/l, which was significantly lower ($p < 0.05$) than the values observed for the other groups. Again, the results showed that the groups fed 0.25 g/l aqueous leaf-extracts of the plant, had total protein values that were significantly higher ($p < 0.05$) than the values obtained for the groups fed commercial growth promoters. Also, it was observed that the groups fed aqueous extracts gave TP values that were significantly higher ($p < 0.05$) than the values obtained in the groups fed ethanol extracts.

The highest albumin value of 4.265 ± 0.064 g/l was obtained at the group fed 0.25 g/l aqueous extracts of *J. secunda*. This value was significantly higher ($p < 0.05$) than the albumin values of the other groups. The negative control group gave the least albumin value of 1.39 ± 0.071 g/l, which was significantly lower ($p < 0.05$) than the values for the groups fed aqueous leaf-extracts of the plants, and the groups fed commercial growth promoters. Again, the results showed that the groups fed aqueous extracts of the plants had significantly higher ($p < 0.05$) albumin values compared to the groups fed ethanol leaf-extracts.

The groups fed 0.25 g/l aqueous leaf-extracts *J. secunda*, with a globulin value of 40.715 ± 0.163 g/l, was observed to have the highest globulin value. The least globulin value was observed at the negative control group with a value of 17.93 ± 0.806 g/l that was significantly lower ($p < 0.05$) than the values obtained for other groups. Again, the results showed that the groups fed 0.25 g/l and 0.50 g/l aqueous extracts of *J. secunda*, had globulin values that were not significantly different ($p > 0.05$) from the groups fed commercial growth promoters.

Hematological indices

It was observed that the hemoglobin values decreased with decreasing concentrations as presented in Figure 3. The highest hemoglobin values were obtained at the group fed 0.75 g/l aqueous extract of *J. secunda* with a value of 9.17 ± 0.707 g/l. The negative control group gave the least hemoglobin value of 6.165 ± 0.233 g/l which was significantly lower ($P < 0.05$) than the values obtained in the groups fed aqueous extract, and the groups fed commercial growth promoters.

Again, the groups fed aqueous extract had significantly higher ($p < 0.05$) hemoglobin values compared to the groups fed ethanol extract of the plant. Similarly, the hemoglobin values of groups fed aqueous extract were not significantly different ($p > 0.05$) from the hemoglobin values of the groups fed commercial growth promoters, whereas the groups fed ethanol extract gave hemoglobin values that were significantly lower ($p < 0.05$) than the values obtained for the groups fed commercial growth promoters.

The highest RBC values were obtained at groups fed 0.75 g/l ethanol leaf-extract of *J. secunda* and 0.75 g/l aqueous leaf-extract of *J. secunda,* with values of 7.85 ± 0.778 $x10^9$/l and 6.7 ± 0.283 $x10^9$/l respectively which were not significantly different ($p > 0.05$) from each other. Again, the negative control group gave the least RBC value of 2.05 ± 0.212 $x10^9$/l which was significantly different ($p < 0.05$) from the values obtained in the groups fed the extracts.

The highest value of WBC was obtained at the negative control group with an WBC count of 18.5 ± 0.707 which was significantly higher ($p < 0.05$) than the values obtained in the groups fed aqueous extracts and the groups fed albiovit. Again, it was observed that the groups fed 0.75 g/l and 0.50 g/l aqueous extracts had significantly lower ($p < 0.05$) WBC count when compared to the groups fed ethanol extracts. Similarly, the groups fed commercial growth promoters gave WBC values that were not significantly different ($p > 0.05$) from the values obtained in the groups fed ethanol leaf-extracts and the groups fed 0.25 g/l aqueous extracts.

The highest PCV was obtained at the group fed 0.75 g/l aqueous extract of *J. secunda* (27.5 ± 0.707). The negative control group gave a PCV of 15 ± 0.001 that was significantly lower ($p < 0.05$) than the values obtained in the other groups. Again, the groups fed aqueous extracts had PCV that were significantly higher ($p < 0.05$) than the values obtained in the groups fed ethanol extracts. Whereas, the PCV of groups fed aqueous extracts were not significantly different ($p > 0.05$) from the PCV of the groups fed commercial growth promoters.

The negative control group had the highest neutrophil value of 10 ± 0.001 which was significantly higher ($p < 0.05$) than the values obtained in the groups fed aqueous extracts and the groups fed commercial growth promoters. The group fed 0.25 g/l aqueous extract of *J. secunda* had the least neutrophil values observed in the study. Nevertheless, there were no significant differences ($p > 0.05$) observed in the neutrophil values of the groups fed aqueous extracts, the groups fed ethanol extracts, and the groups fed commercial growth promoters.

The various treatment groups gave eosinophil values that were dependent on the dose of the leaf-extracts administered. The groups given aqueous extracts had eosinophil values that increased with decreasing concentrations, whereas, the groups fed ethanol extracts gave eosinophil values that increased with increasing concentrations. It was observed that the negative control group had the highest eosinophil value of 5.0 ± 0.001 which was significantly different ($p < 0.05$) from the values obtained in the other groups. Moreover, it was observed that the eosinophil values of groups fed aqueous extracts were not significantly different ($p > 0.05$) from the values of the groups fed ethanol extracts and the groups fed commercial growth promoters.

The various treatment groups gave lymphocyte values that were dependent on the dose of the extracts administered as it increased with the decreasing concentrations of the aqueous extract, but decreased with the decreasing concentration of the ethanol extract. It was observed that the negative control group had the highest lymphocyte value of 5.0 ± 0.001 which was significantly higher ($p < 0.05$) than the values

obtained for the other groups. Again, the lymphocyte values of the groups given aqueous extracts were not significantly different ($p < 0.05$) from the lymphocyte values of the groups fed commercial growth promoters and the groups fed ethanol extracts.

Again, Table 4 shows that all the blood components measured were affected by the extracts administered to the chickens. A component plot of blood parameters in rotated space (Figure 4) show that all the data appear normal and no extreme outliers were apparent.

Table 4

Communalities (principal component analysis) of blood parameters.

	Initial	Extraction
Hb	1.000	.891
RBC	1.000	.922
WBC	1.000	.890
Neutrophil	1.000	.787
Eosinophil	1.000	.838
Mesophil	1.000	.928
PCV	1.000	.956
Lymphocyte	1.000	.821
Total protein	1.000	.963
Globulin	1.000	.952
Albumin	1.000	.911

Variables having **high communalities** (r^2 value, -say more than 0.70) contributes more to measuring the underlying principle components (factors). Hence, by implication, all parameters having $r^2 \geq 0.7$ is said to have been highly affected by the treatment across the groups.

Discussion

The results of vitamin composition of the aqueous and ethanol extracts of *J. secunda*, as demonstrated in Table 1, show that these plants contain water soluble vitamins (C and B-complex) and fat-soluble vitamins (A, E, and D) in different proportions. The vitamin A content of the plants are above the minimum requirement of 54µg/100g in broiler diets (Ogunmodede, 1981) implying that the extracts are good sources of vitamin A. This vitamin is important in broiler production as it serves as a free radical scavenger, as well as promotes growth and healthy eyes (Pal, 2017).

The vitamin E content of the plants were below the daily requirements of 5–10 mg/kg by National Research Council (1994) in poultry production. However, the vitamin E amounts present in the main ingredients utilized in poultry diets are sufficient for the poultry need (Pompeu *et al.*, 2015) thereby nullifying any negative effect that the low vitamin E content of the extracts could have posed if used as growth promoter. The contribution of this study is obvious as the outcomes show that the plants can help to increase the vitamin E available for broiler intake and subsequently improve growth performance, carcass yield, maintain bone structure and meat quality of the birds (Pal, 2017).

On the other hand, the vitamin D content of the plant was well above the 5 µg/kg of feed recommended by the National Research Council (1994), but falls within the suggestion of European Union that the current legal maximum dietary limit of vitamin D in poultry could be 125 µg/kg of feed (Sakkas *et al.*, 2019). The findings therefore, provide evidence that the plant is a good source of vitamin D in poultry production. Vitamin D is important in broiler production for skeletal integrity through the stimulation of the expression of genes in the small intestine which govern the absorption of intestinal calcium and phosphorus, in bone to osteoclast differentiation and calcium reabsorption promoting mineralization (St-Arnaud, 2008). Also, the supplementation of vitamin D in broiler diets enhances the breast meat yield and quality (Vignale *et al.*, 2015).

The functions of minerals in poultry production cannot be over emphasized. Minerals are implicated in the formation of the skeleton, function as cofactors of enzymes and are important for the maintenance of osmotic balance within the bodies of birds (National Research Council, 1994). Table 2 demonstrates that the plants under study contain some macro elements such as calcium, phosphorus, potassium, sodium, magnesium, and micro elements such as copper, iron, manganese, selenium, and zinc which are necessary for broiler production. These findings are consistent with the findings of Arogbodo (2020) and Chandran (2017), who concluded in their individual researches that this plant contain these elements in different proportions. The presence of these elements in the extracts implies that the plants can contribute in increasing their availability for broiler consumption. A deficiency in anyone of the essential minerals can result in chronic metabolic disorders which can hamper the health of the birds (Chandran, 2017).

Again, this study provide evidence that the plants contain some heavy metal group of elements such as nickel and arsenic. Nevertheless, the levels of arsenic in the extracts were below the maximum permissible limit of arsenic, 30 ppm and 2 ppm by European Commission (2003) and National Research Council (2005) respective, in poultry feeding stuff. This is an indication that the plants under study, are safe sources of the element for broiler consumption. Balos *et al.*(2019) reported that the presence of arsenic in poultry diet is essential for the synthesis of methionine metabolites such as cysteine.

Again, the mean amount of nickel is well below the 250 ppm permissible mean concentration set for nickel by National Research Council (2005). The significance of this finding is that the presence of nickel in *J. secunda* is an indication that the plant can contribute in increasing yield in broilers. A deficiency of

nickel in poultry diet have been reported to contribute to retardation of growth, reduced bone strength, joint swelling, shortening and thickening of leg bones (Balos *et al.*, 2019).

The presence of flavonoids is the major contributor for the anti-inflammatory, anti-oxidant and anti-nociceptive properties of *J. secunda* (Onoja et al., 2017). Also, flavonoids have been associated with possible roles in the prevention of several chronic diseases involving oxidative stress in poultry and other animals (Shanaz *et al.*, 2011). Recent evidence suggests that increasing the levels of flavonoids in broiler diet leads to an improvement in growth performance, blood constituents, carcass composition and small intestinal morphology (Prihanbodo *et al.*, 2021). Kamboo *et al.*(2016) concluded in their study that supplementation of plant flavonoids generally improves the immunity and antioxidant status of growing broilers.

In the same vein, the high anthocyanin observed in ethanol extract of *J. secunda* is in line with earlier literatures that the plant is taken in cases of amenorrhea and anemia (Mea *et al.*, 2017). These assertions reflect the findings of Mpiana *et al.*(2010) who concluded in their study that anthocyanin stabilizes the red blood cells through decreasing intercellular hemoglobin concentrations and inhibiting cell dehydration. Again, Osioma and Hamilton-Amachree (2017) reported that the antioxidant activities of *J. secunda* could be attributed to the presence of saponins, phenols, and other polyphenols they contain.

The findings of this study show that the body weight gain of the broilers was significantly improved by the extracts. It was observed that 0.50 g/l and 0.75 g/l of aqueous extracts improved the weight gain of the broilers better than the ethanol extracts. Compared to the control groups, the extracts produced weight gain values that were not significantly different from the groups fed commercial growth promoters, but improved the broiler weight gain better than the negative control group. The good performance of the broilers fed the extracts may be due to the presence of vitamins, minerals and other phytochemicals in the extracts, which boost growth. Reports have shown that feeding broiler chickens with diet containing phytogenic blend leads to improvement in body weight gain (Mohammadi-Gheisar et al., 2015)

In the same vein, another striking result to emerge from this study is that the specific growth rate of the groups fed the leaf-extracts were significantly higher ($p > 0.05$) than the specific growth rate of the negative control group. Also, the findings revealed that the specific growth rate of the groups fed 0.5 g/l and 0.75 g/l aqueous extracts were significantly ($p < 0.05$) higher than the values of the groups fed ethanol extracts. These observations are supported by the reports of Hernandez et al. (2004) who concluded that broilers fed normal diet plus plant extracts grow faster than those fed normal diet alone. Similarly, Jayanti *et al.*(2017) observed that supplementation of *Moringa oleifera* leaf powder (a phytogenic growth promoter) in broiler diets improves growth performance. Among the plausible explanations for these findings could be due to the presence of metabolites such as vitamins, minerals and other phytochemicals contained in these extracts.

Again, the results revealed that there were no significant differences ($p > 0.05$) between the treatment groups in their drip losses and cooking losses of the broilers. This is consistent with the findings of Oko *et al.*(2013) who found that feeding broilers with *Mucuna poggei* (a phytogenic growth promoter)

produces no significant difference ($p > 0.05$) in the mean drip losses of the birds. Nevertheless, the drip loss and cooking loss of the negative control group was significantly higher ($p < 0.05$) than the other groups. This suggests that the meat of broilers fed with the extracts is of high quality because little or no nutrient was loss in the cooking water. Protein is lost into the water due to proteolysis (Oko *et al.*, 2013). The findings of the present study agree with Oko et al. (2012) and Oko (2013), that had earlier reported that meat with low cooking loss has higher meat quality and protein content.

The concentrations of total protein and globulin in the serum of the broilers were significantly increased ($p < 0.05$) by the extracts compared to the negative control. These results are in agreement with the findings of Zhang et al. (2009), who observed increased total protein and globulin contents in the plasma of broilers fed with ginger powder. The group fed aqueous extracts were observed to have significantly higher total protein and globulin compared to the groups fed ethanol extracts. This observed increase reflects the ability of chicks fed with these extracts to store excess protein even after the body has reached its maximum capacity for depositing proteins to tissues (Ghazalah and Ali, 2008).

Similarly, there was a significant increase ($p < 0.05$) in serum albumin values of the groups fed aqueous extracts compared to the negative control group. These results reflect the reports of Abd El-Hady et al. (2020) who opined that phytogenic extracts have a positive effect on serum albumin of broilers. The observed high content of albumin in blood serum of broilers also indicates enhanced nutrient supply and transport (Ghazalah and Ali, 2008).

Hematological indices have been recognized as one of the indicators for assessing the health status of animals (Oloruntola *et al.*, 2016). The effects of the extracts on blood hematology parameters showed that the groups fed aqueous extracts had significantly higher ($p < 0.05$) hemoglobin and packed cell volume compared to the negative control group. But the hemoglobin and packed cell volume of the groups fed aqueous extracts were not significantly different ($p < 0.05$) from the results of the groups fed commercial growth promoters. These values recorded for hemoglobin and packed cell volume in the broilers fed aqueous extracts and commercial growth promoters indicate nutritional adequacy and improvement / stability of their hematological profile (Oloruntola, 2019).

Red blood cell is responsible for the transportation of oxygen and carbon dioxide in the blood as well as manufacture of hemoglobin. So, higher values indicate a greater potential for this function and a better state of health (Olugbemi *et al.*, 2010). An increase was recorded in the red blood cell count of the groups fed 0.50 g/l and 0.75 g/l extracts compared to the control groups. The red blood cell count increased with the increasing concentration of the extracts. This finding is consistent with earlier literatures that the inclusion of leaf-extracts such as *Ipomoea asarifolia* leaf (Madubuike and Ekenyem, 2006), *Teifaria occidentalis* (Nworgu *et al.*, 2007) in broiler diets have resulted in significant increase in red blood cell counts. The results of this study tend to evidence that this plant possesses such blood tonic effects.

The white blood cells, consisting of neutrophils, eosinophils, lymphocyte and basophils are involved in protecting the body against infections. They destroy virus-infected cells, and enhance the production of antibodies. A high concentration of differential white blood cells in the body connotes a threat to normal

health, therefore the body builds up its defense against such threat (Olugbemi *et al.*, 2010). So, it is apparent from Figs. 12, 13, 14 and Table 4 that these extracts, especially the aqueous extracts, improved the ability of the bird to fight infections, defend their bodies against foreign organisms' invasion, and to produce and distribute antibodies. This is because the extracts were able to significantly lower ($p < 0.05$) the total white blood cell count, neutrophil, eosinophil and lymphocyte levels of the broilers compared to the negative control. Finally, the treatment groups were comparable to the groups fed commercial growth promoters as there were no significant differences ($p > 0.05$) recorded.

Conclusion

This study set out to determine possible use of crude leaf-extracts of *J. secunda* as growth promoters in poultry industries. It was observed that the extracts enhanced broilers growth and improved their meat quality. Also, the hematological and serum biochemical parameters were found to be improved within normal ranges suggesting safety of the extract at the given concentrations. The findings suggest that in general the aqueous and ethanol leaf-extracts of the plant can serve as replacements for chemical growth promoters. However, the aqueous extract produced better effects than the ethanol extract. Therefore, the current findings add substantially to our understanding of the importance of the extracts and why the plant should be exploited as alternative to chemical growth promoters used in broiler industries.

Declarations

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Authors contributions

Conceptualization: AOO, ETE; Material collation: AOO, ETE; Methodology: AOO, ETE; Statistical analysis: SE, ETE; Resources: AOO, ETE; Supervision: AOO, SE; Writing – Original draft preparation: ETE; Writing – Review and Editing: AOO, ETE, SE

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Declarations

Ethics and animal welfare

Animal care and procedures were performed by following the guidelines of good experimental practices. The Ebonyi State University Ethical Committee granted permission for this research to be conducted.

Consent for participation

Informed consent was obtained from all individual participants included in the study.

Consent to publish

Not applicable.

Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

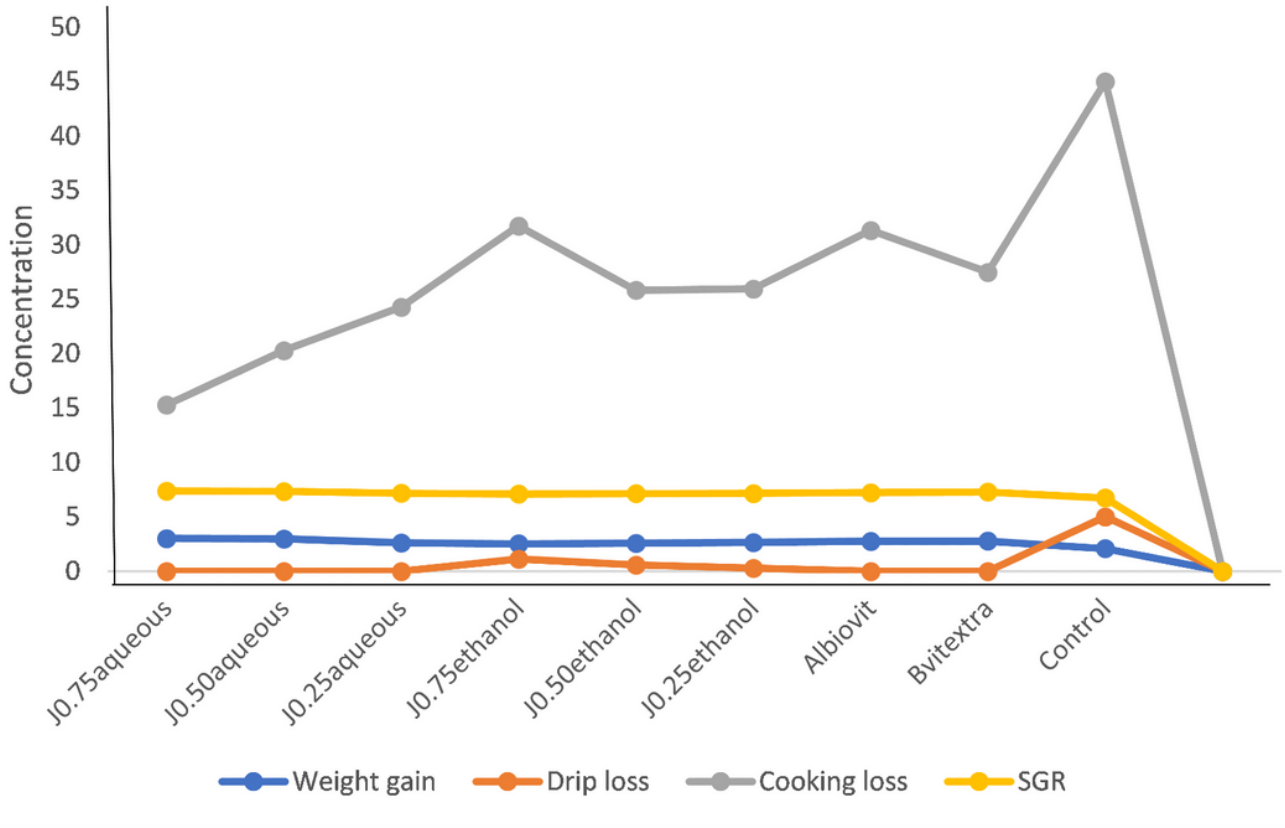

Figure 1: Effects of the aqueous and ethanol leaf-extracts on the broiler carcasses. 'J' represents *J. secunda*

Figure 1

See image above for figure legend.

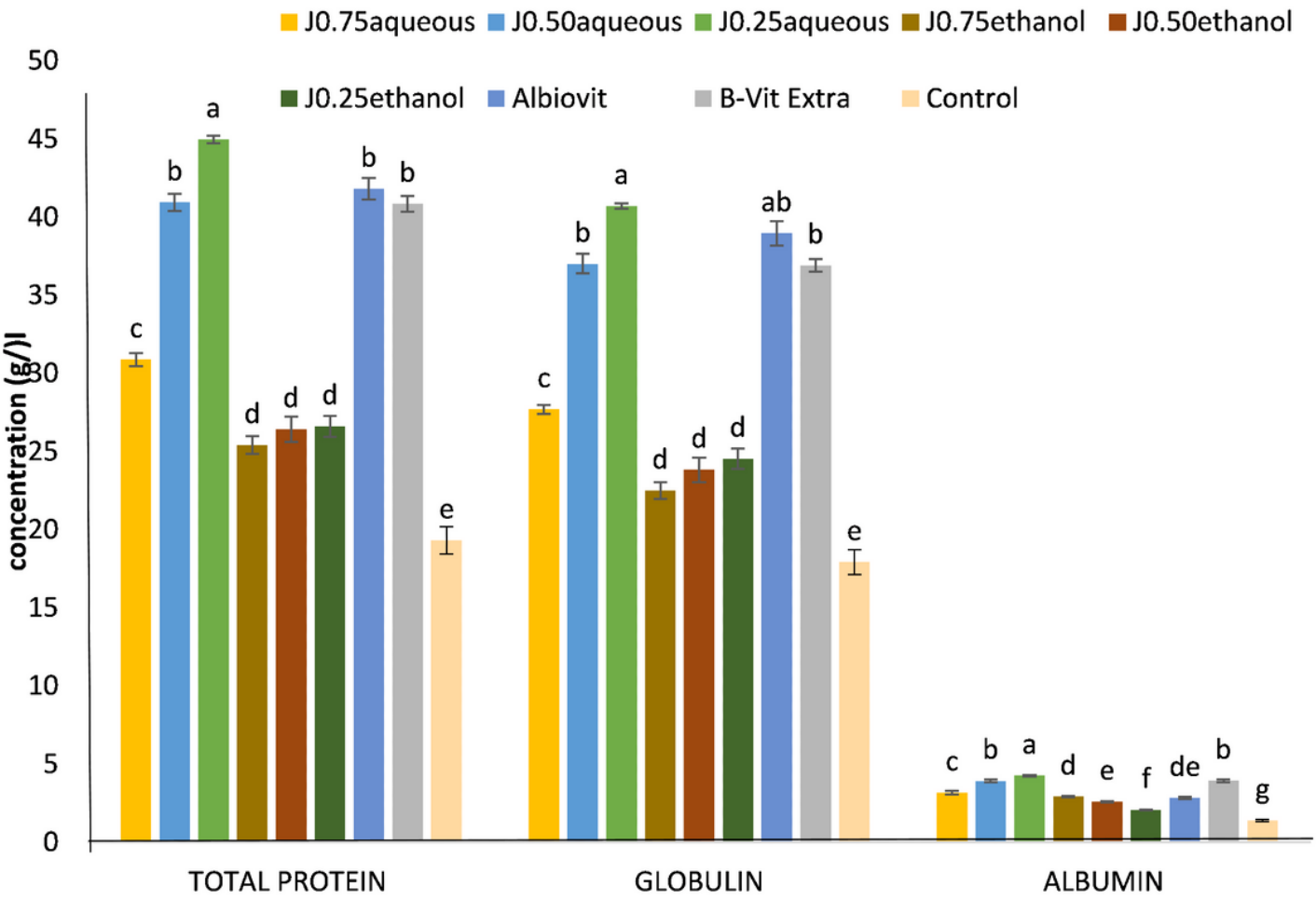

Figure 2: Effects of the aqueous and ethanol leaf-extract on the blood protein concentrations of the broilers. 'J' represents *J. secunda.* Values are presented as mean±SD.

Figure 2

See image above for figure legend.

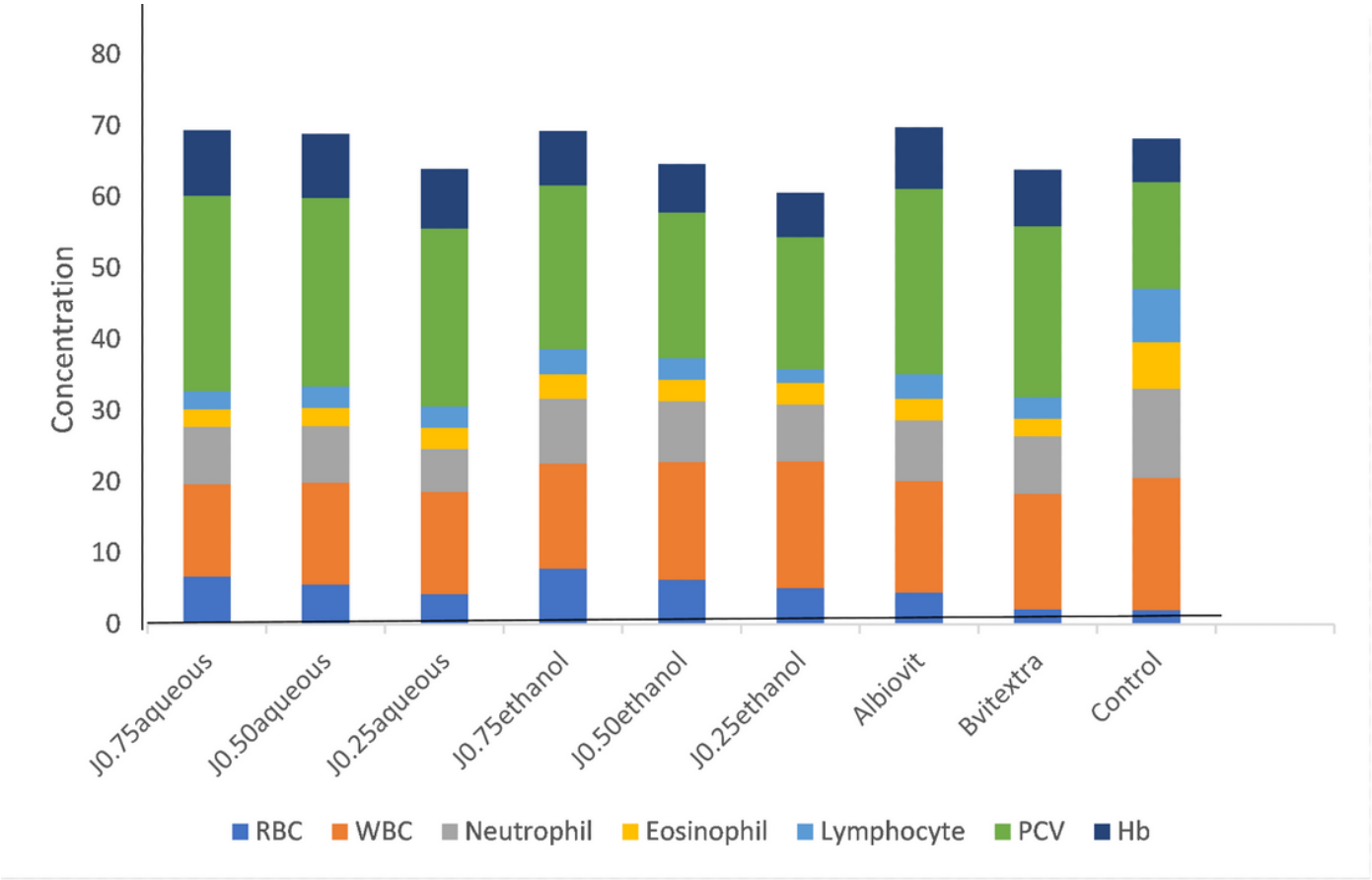

Figure 3: Effects of the aqueous and ethanol leaf-extracts on the hematological parameters of the broilers. 'J' represents *J. secunda*

Figure 3

See image above for figure legend.

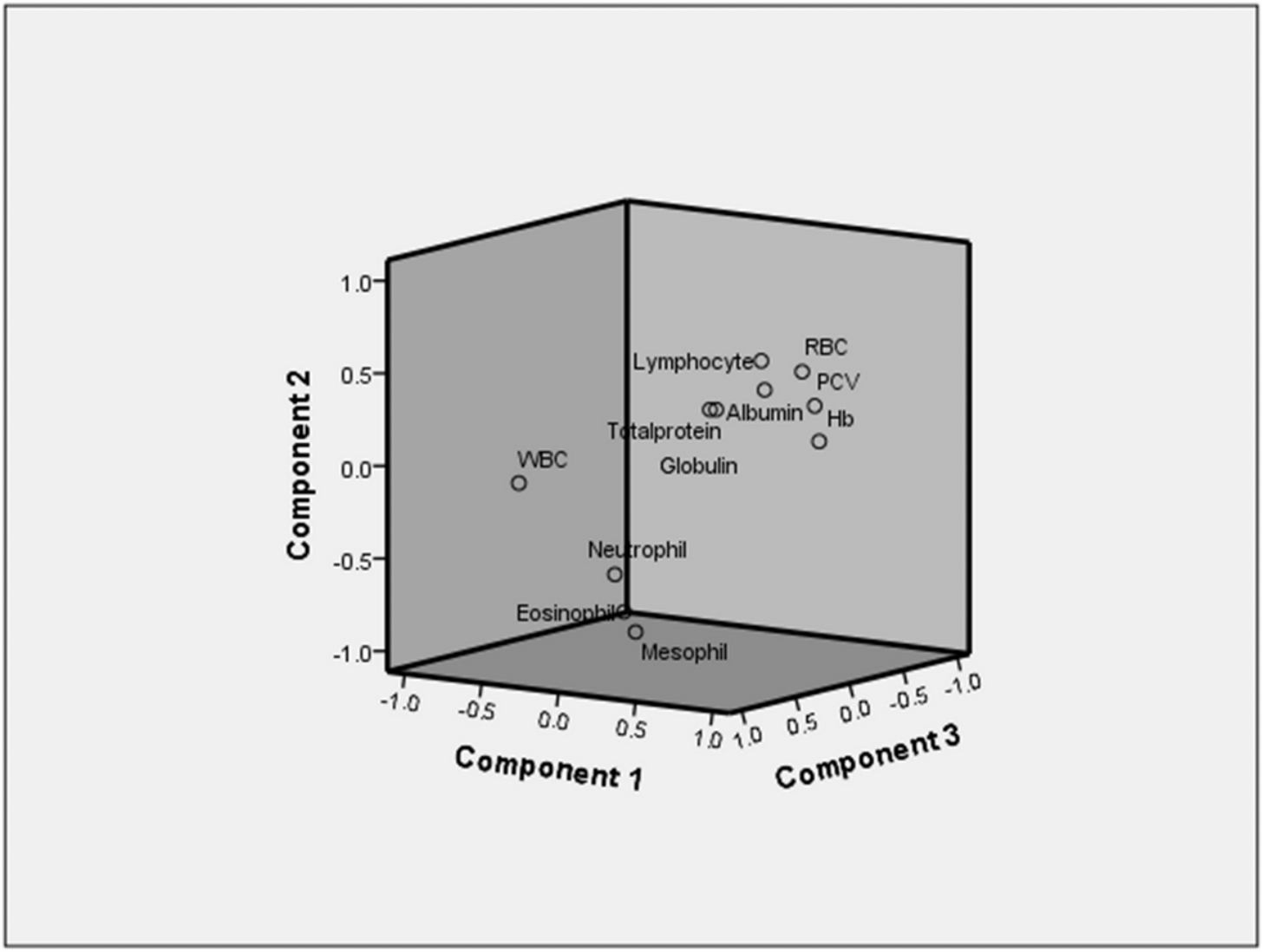

Figure 4: Component plot of blood parameters in rotated space. The data appear normal and no extreme outliers are apparent.

Figure 4

See image above for figure legend.