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Evaluation of *Justicia secunda* as broiler phytogenic growth promoter

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Abstract
Objective: Aqueous and ethanol leaf extracts of *Justicia secunda* (Vahl) were studied for application as growth stimulants in broilers.
Methods: Two hundred and eighty-eight (288) day-old-broilers (Ross 308) (48 ± 1 g) were purchased, randomly grouped into 9 treatments with 4 replicate pens containing 8 birds per pen. Treatments applied to the groups include basal diet, basal diet plus 0.75 g/kg aqueous leaf-extract/kg feed, basal diet plus 0.5 g/kg aqueous leaf-extract/kg feed, basal diet plus 0.25 g/kg aqueous leaf-extract/kg feed, basal diet plus 0.75 g/kg ethanol leaf-extract/kg feed, basal diet plus 0.5 g/kg ethanol leaf-extract/kg feed, basal diet plus 0.25 g/kg ethanol leaf-extract/kg feed, basal diet plus 0.5 g/kg Albiovit/kg feed, and basal diet plus 0.5 g/kg B-vit-extra/kg feed. The experiment lasted for 56 days. Growth performance, serum biochemistry, and carcass yields of the broilers were verified by standard methods.
Results: Results indicated that broilers given diets containing the extracts showed a marked increase in growth performance from 28 days. At 28, 42, and 56 d of age, broilers that received an inclusion of 0.75 g aqueous extract had the highest body weight of 886.7±17.96, 1859.1±16.83, and 2920±28.28, respectively, compared with the other groups. In contrast, the broilers that received only a basal diet had the lowest body weight.
Conclusion: These results suggest that *J. secunda* leaf extracts can replace chemical growth stimulants, although the effects of the aqueous extracts were more significant on the broilers compared to the ethanol extracts.

Keywords: *Justicia secunda,* Growth stimulant, Growth performance, Phytogenic, broilers

Plain English Summary

The use of antibiotics as growth promoters in the broiler industry has become a global concern. Although antibiotics help improve broiler growth and general health, their persistent use results in their accumulation in the bodies of broilers resulting in antibiotic resistance in broilers, and subsequent transmission of antibiotic-resistant plasmids and or strains of organisms incorporating such plasmids, to humans and nearby aquatic and terrestrial environments. To this effect, there is a global shift to less toxic alternatives, especially of plant origin. Therefore, this work set out to study *J. secuda* leaf as a possible alternative to antibiotics in broiler production. First, the mineral, vitamins, and phytochemical

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compositions of the leaf were studied. Then, the leaf was used to grow 288 1-day-old broilers for 56 days. This was done by including the aqueous or ethanol extracts of the leaf in broiler diets. Afterwards, growth performance, serum biochemistry, and carcass characteristics of the broilers were studied. At the end of the experiment, it was discovered that the leaf extracts improved the broiler growth performance, and serum biochemistry but had no visible impact on their carcass characteristics. Thus, it was concluded that ethanol and aqueous leaf extracts of *J. secuda* could serve as an alternative to antibiotics in broiler production, with the aqueous extract as the preferred alternative.

Background

Broiler production is targeted at achieving optimal production at the lowest possible cost to ensure that the growing demand for animal protein is met as well as provide humans with safe and additive-free food. The use of novel growth stimulants like VITA nutrition, polamix, B-vit extra, and Albiovit, in Africa, particularly Nigeria, has led to unprecedented growth in the broiler sector (1). These chemicals have been primarily exploited by broiler farmers to enhance growth, health, and overall poultry performance. Over time, their prolonged use, particularly those that contain antibiotics, is known to have adverse effects on poultry, resulting in the development of antibiotic resistance and subsequent chemical residue accumulation in the animals, which can be transmitted to humans (2). According to studies, drainage is a crucial pathway for toxic substances entering the environment as chemical growth promoters used for poultry can be excreted through urine and faeces. Nearby farms and aquatic ecosystems such as lakes and ponds can be contaminated by these excreta (3, 4). The poultry industry therefore faces an increasing demand for superior growth stimulants (mineral and vitamin supplements) that can promote the growth of the birds as well as improve their overall performance without much competition with the supply chain (5). In this light, it is necessary to look for cheaper, locally produced alternatives that are competitive with available chemical growth stimulants.

Poultry researchers have been testing other possible substitutes – including probiotics, prebiotic substances, herbs, and organic acids - that could help to improve the overall poultry health and productivity while being harmless to both the birds and humans (6, 7). Phytobiotics, dietary supplements of plant origin, have been resorted to as preferred choices over chemical growth enhancers. Phytobiotics possess numerous therapeutic properties and a low incidence of adverse impact (8, 9). In poultry farming, phytobiotics, are essential as they aid in appetite stimulation, endogenous digestive secretions enhancement, and immune response stimulation, resulting in the production of quality poultry products (7). The plant, *J secunda*, containing several pharmacologically active compounds, may belong to this group.

J. secunda, a perennial herbaceous plant belonging to the Acanthaceae family, is indigenous to Africa and Asia and is predominantly grown as a garden ornament in Southeast Nigeria (10). It is commonly known as bloodroot in Africa ("sanguinaria" in Venezuelans, "ewe eje" in Yoruba, or "ogwu obara" in Igbo) and the Jehovah's Witnesses in Southern Nigeria, Congo, and Southern Ivory Coast have been known to decline blood transfusions, resorting to *J. secunda* leaf for the treatment of anemia (11). Venezuelans employ *J. secunda* as an antipyretic agent as well as a cure for chickenpox (11). Other folkloric uses of this plant in Africa include wound healing, anaemia, and abdominal pain (12, 13). A range of phytogenic compounds, including tannins, flavonoids, alkaloids, phenols, and vitamins have been reported for this plant (10), which lends weight to some of its characteristic pharmacological properties (antiviral, anticancer, antibacterial, antisickling, antiinflammatory and antiplatelet properties) (14). This study was therefore aimed at evaluating *~~Justicia~~ secunda* as a broiler phytogenic growth promoter feed inclusion.

Materials and Methods

Study location

The study site was situated at a poultry unit of the Teaching and Research Farm of the Department of Animal Science, Ebonyi State University Abakaliki, which is located in the university CAS campus, Abakaliki. Abakaliki is located within the South-eastern guinea savannah ecological zone between latitude 8° 30′ and 9° 40′ North and longitude 5° 40′ and 6° 45′ East.

Preparation of J. secunda leaves

The mature leaves were harvested from the plant's stem during the rainy season (18th June 2020) from Ehugbo, Afikpo Local Government Area, Ebonyi State, Nigeria. The leaves were authenticated by a taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki (Voucher number: EBSU-H-809) and a sample was deposited in the herbarium unit of the Department. The clean leaves were air-dried until crispy on the touch while retaining their colouration. The leaf samples were pulverized in a manual Corona blender. Both ethanol and aqueous extractions were performed.

Extraction

Aqueous extract was prepared using the method of Soetan *et al.* (15), whereas, the methods of Asuzu and Onu (16) and Builders *et al.* (17) were used in preparing the ethanol extract using the Soxhlet apparatus. Each of the ethanol and aqueous extracts was independently concentrated at a reduced temperature (45 ^{0}C) in a water bath, and an airtight container (4 ^{0}C) was used to store the extract yield (w/w) as described by Josiah *et al.* (18).

Vitamin analysis

The methods of Kirk and Sawyer (19) were used for the determination of the various vitamins.

Minerals analyses

An atomic absorption spectrometer (Agilent FS240AA, United States of America) was used to determine the mineral content of the leaves according to the method of APHA (20).

Phytochemical evaluation

One gram of the sample was measured into a sterile test tube and ethanol (15 mL) and potassium hydroxide (10 ml of 50% w/v) were added to the tube. The tube was placed in a water bath (60 °C) to react for 1 h. After the initial reaction, the sample was collected into a separating funnel and washed three times with ethanol (20 ml), warm water (20 mL), and hexane (3 mL). Anhydrous sodium sulfate was used to dry the solution, after which the solvent was evaporated. Thereafter, 1000 µL of pyridine was used to dissolve the sample before collecting 200 µl of the mixture for analysis. A gas chromatograph (BUCK M910, United States of America) equipped with a flame ionization detector (GC-FID) was used for the analysis. The GC-FID uses Helium 5.0 pa.s as carrier gas with a flow rate of 40 ml/min, operating at a temperature and speed of 280°C and 30 cm/s respectively. The concentration of phytochemicals (µg/mL) was determined as the surface area of the mass of the internal standard to the total surface area of each phytochemical species. The phytochemicals analyzed include: flavan-3-ol, flavone, flavonones, phenol, proanthocyanin, oxalate, sapogenin, phytates, tannins, kaempferol, steroids, ribalinidine, epicatechin, ephedrine, naringenin, rutin, lunamarin, quinine, and spartein.

Acute toxicity test

The up-and-down acute toxicity method was used to conduct this study (21).

Animals and dietary treatment

This study targeted the examination of the effect of the extracts of *J. secunda* in comparison to commercial feed additives (Albiovit and Bvitextra) on chicken growth performance, carcass trait, and serum biochemistry. A total of 288, 1-day-old broilers (Ross 308) were purchased, weighed (48±1 g) and were randomly allotted to 9 groups of 4 replicate pens with 8 chicks per replicate pen. The groups were treated as follows: a basal diet (negative control), basal diet plus 0.75 g aqueous extract/kg of diet, basal diet plus 0.5 g aqueous extract/kg of diet, basal diet plus 0.25 g aqueous extract/kg of diet, basal diet plus 0.75 g ethanol extract/kg of diet, basal diet plus 0.5 g ethanol extract/kg of diet, basal diet plus 0.25 g ethanol extract/kg of diet, basal diet plus 0.5 g Albiovit/kg of diet and basal diet plus 0.5 g B-vit-extra/kg of diet. A deep litre pen (120 cm x 120 cm x 80 cm) was the base on which the birds were raised for 56 days, in a room that had optimum environmental conditions. There was adequate feed and water provided for the broilers *ad libitum* throughout the experimental period. There was continuous lighting by incandescent bulbs for the first week to provide an initial temperature of 33 ^{0}C. The temperature was gradually reduced by 3 ^{0}C per week before fixing it at 21 ^{0}C.

Performance and carcass component

Evaluation of the body weight (BW) was done on days 1, 14, 28, 42, and 56. Average daily weight gain (DWG), and daily feed intake (DFI) were both determined for these periods and the whole period of the experiment. DFI was corrected for dead broilers. DFI to DWG ratio was used in the calculation of the feed conversion ratio (FCR) (22).

On the 56th day, 2 broilers were selected following the average body weight of the pen. The weights of the selected birds were taken before slaughtering them by cutting the jugular vein. Evaluation of the carcass yield was by the percentage of the eviscerated weight to the live weight. The empty small intestine, liver, kidney, and heart were removed from carcasses, their weights were taken, and their yield was calculated as the percentage of their weight to live weight (22).

Biochemical analysis of blood

At 52 d of age, the birds were fasted for 12 h before blood samples of approximately 2 ml were collected from 2 broilers in each replicate from the brachial vein using vacuum tubes. The blood samples were placed in plain containers for serum biochemistry. The blood samples were left to clot and retract at a temperature of 37 ^{0}C for 2 h, and centrifuged (at 2000 x g) for 10 min (SIGMA 4e15 Lab Centrifuge, Germany). The sera were separated and analyzed for total protein and albumin using commercial kits (Randox Laboratories, United Kingdom) under strict compliance with the manufacturer's instructions. The globulin was estimated by subtracting albumin concentration from total protein.

Statistical analysis
Data were analyzed using Statistical Package for Social Sciences (SPSS®) for Windows® version 23. Duncan Multiple Range Test was used to compare the differences between means ($p < 0.05$) (23).

Results

Vitamins contained in the extracts
Table 1 shows the concentrations of the various vitamins (A, E, C, D, B1, B2, B3, B6, and 12) as observed from the study. Aqueous leaf extract had values of vitamins (E, C, D and B_3) that were significantly different ($p < 0.05$) from the values observed for the ethanol extract. However, the values of vitamins (A, B_1, and B_6) contained in the aqueous leaf extract were not significantly higher ($p > 0.05$) than the values observed for ethanol extracts. The aqueous leaf extract had a higher concentration of vitamin C than the ethanol extract, whereas, the ethanol extract had higher concentrations of vitamins (A, E, and D) than the aqueous extract.

Table 1: Result of the water- and fat-soluble vitamins contained in the extracts

Vitamins	Ethanol extract	Aqueous extract	P
Vitamin A (µg/g)	73.64±0.36	72.76±0.2	0.013
Vitamin E (µg/g)	68.21±0.32	29.73±0.58	0.000
Vitamin C (µg/g)	87.10±0.2	88.12±0.2	0.002
Vitamin D (µg/g)	38.36±0.35	24.03±0.26	0.000
Vitamin B_1 (mg/g)	0.17±0.07	0.16±0.003	0.807
Vitamin B_2 (mg/g)	0.21±0.01	0.16±001	0.001
Vitamin B_3 (mg/g)	4.39±0.11	3.59±1.05	0.005
Vitamin B_6 (mg/g)	0.60±0.1	0.63 ±0.04	0.767
Vitamin B_{12} (mg/g)	0.05±0.01	0.16±0.004	0.012

Minerals contained in the extracts
The mineral composition of the extracts is presented in Table 2. The extracts contained macro elements (Magnesium, calcium, potassium, sodium, phosphorus, iron, and zinc), and trace elements (nickel, arsenic, manganese, molybdenum, selenium, copper, and cobalt). Manganese and cobalt were observed in the aqueous leaf extract but were not present in ethanol leaf extract. The macro elements and trace elements contained in the aqueous leaf extract were significantly higher ($p<0.05$) than the values obtained from the ethanol leaf extract.

Table 2: Result of the minerals contained in the extracts

Minerals	Ethanol extract	Aqueous extract	P
Nickel (mg/g)	0.001±0.002	0.007±0.002	0.001
Magnesium (mg/g)	5.612±0.007	5.337±0.04	0.000
Molybdenum (mg/g)	0.028±0.002	0.043±0.003	0.000
Arsenic (mg/g)	0.062±0.002	0.083±0.002	0.000
Zinc (mg/g)	1.442±0.036	1.989±0.092	0.000
Calcium (mg/g)	8.084±0.059	0.473±0.002	0.000
Cobalt (mg/g)	NIL	0.117±0.002	0.000
Potassium (mg/g)	9.071±0.064	8.888±0.04	0.000
Manganese (mg/g)	NIL	0.206±0.005	0.000
Copper (mg/g)	0.147±0.005	0.135±0.004	0.004
Selenium (mg/g)	0.488±0.008	0.642±0.002	0.000
Sodium (mg/g)	3.92±0.011	2.289±0.003	0.000
Phosphorus (mg/g)	0.009±0.002	0.001±0.002	0.000
Iron (mg/g)	5.002±0.002	5.183±0.1	0.002

Phytochemicals contained in the extracts
The phytochemical composition of the extract is indicated in Table 3. The plant extract was found

to have flavanone, flavan-3-ol, flavone, tannin, phenol, and oxalate, as well as various other compounds such as sapogenin, kaempferol, steroid, epicatechin, ephedrine, naringenin, and lunamarin. The study also revealed that both extracts contained phytate. The values of these metabolites (flavones, phenols, sapogenins, tannins, anthocyanins, steroids, and quinine) were not significantly different (p > 0.05) for both extracts. Similarly, significantly (p < 0.05) high values of flavan-3-ol, flavanone, phenol, sapogenin, phytate, and steroids were contained in the aqueous extract compared to the ethanol extract.

Table 3: Result of the phytochemicals contained in the extracts

Phytochemicals	Ethanol extract	Aqueous extract	P
Flavan-3-ol (µg/mL)	13.879±0.185	20.796±0.1	0.000
Flavone (µg/mL)	17.88±0.287	5.97±0.351	0.000
Flavonones (µg/mL)	11.293±0.249	12.672±0.44	0.002
Phenol (µg/mL)	9.359±0.327	18.763±0.159	0.000
Proanthocyanin (µg/mL)	7.412±0.165	7.963±0.186	0.070
Oxalate (µg/mL)	7.728±0.333	6.655±0.425	0.007
Sapogenin (µg/mL)	11.407±0.463	24.195±0.271	0.000
Phytate (µg/mL)	NIL	11.234±0.336	0.000
Tannins (µg/mL)	34.041±0.409	13.665±0.451	0.000
Kaempferol (µg/mL)	28.596±0.331	17.072±0.233	0.000
Steroids (µg/mL)	4.259±0.418	8.815±0.219	0.000
Ribalinidine (µg/mL)	18.99±0.126	15.748±0.388	0.000
Epicatechin (µg/mL)	2.832±0.343	3.784±0.637	0.045
Catechin (µg/mL)	NIL	25.538±0.529	0.000
Epihendrine (µg/mL)	9.393±0.361	6.629±0.515	0.000
Naringenin (µg/mL)	6.802±0.243	20.521±0.373	0.000
Rutin (µg/mL)	14.434±0.428	11.492±0.583	0.000
Lunamarin (µg/mL)	12.223±0.098	10.275±0.286	0.000
Quinine (µg/mL)	4.663±0.263	12.23±0.322	0.000
Spartein (µg/mL)	3.751±0.213	NIL	0.000

Acute toxicity

After 48 hours, no death or toxic effects were observed after consuming 2.0 g/L of the leaf extract and the same amount of pure distilled water. The concentration of the extracts did not exceed 2.0 g/L as this was already far above the required concentration for the experiment.

Performance and carcass components.

Table 4 shows the summary of performance indices. At 14 d of age, no marked effects of dietary treatments were noted on the broilers. At 28, 42, and 56 d of age, supplementing the broilers-fed diets with ethanol extract resulted in a decrease in the body weights of the birds, which were significantly lower (p<0.05) compared with the body weights of broilers fed diet supplemented with aqueous extract, but did not differ significantly (p>0.05) from broilers fed diets supplemented with the commercial growth promoters (Albiovit and Bvitextra). At 28, 42 and 56 d of age, broilers receiving 0.75 g aqueous extract had the highest body weight (88.7 g, 1859.1, 2920 g respectively) compared with the other groups, while the broilers that received only basal diet without any supplementation (negative control) had the lowest body weight (588 g, 1101 g, 1970 g respectively) compared with the other groups (p<0.05).

At 1-14 d of age, daily feed intake (DFI) did not differ between the treatment groups (p>0.05). At 15-28 d of age, DFI did not differ significantly across all the groups except for the group fed only basal diet (negative group) which had DFI that was significantly lower (p<0.05) compared with other groups. At 29-42 d of age and 43-56 d of age, groups fed diets containing aqueous extracts had higher DFI (p<0.05) compared with other groups. In the whole experiment, 1-56 d of age, broilers fed diets containing aqueous extract had higher DFI compared with broilers fed diets containing ethanol extract, whereas broilers fed diets containing commercial growth promoters (Albiovit and Bvitextra) had DFI that had no significant difference (p>0.05) from broilers fed diet containing aqueous extract and broilers fed diet containing ethanol extract.

At 1-14 d of age, the feed conversion ratio (FCR) of the broilers across all groups was not significantly different (p>0.05). At 15-28 d of age, the differences in the FCR of broilers fed diets supplemented with commercial growth promoters (Albiovit and Bvitextra) and broilers fed diets supplemented with ethanol extract or aqueous extract were not significant (p>0.05). At

29-42 d of age and 43-56 d of age, FCR did not differ across all the treatment groups. At 1-56 d of age, broilers fed commercial growth promoters (Albiovit and Bvitextra) had FCR that were not significantly different (p>0.05) from the FCR of broilers fed diets supplemented with ethanol extract and those supplemented with aqueous extract.

Table 4: Effect of dietary treatments on performance indices of broilers at different ages

Item	Experimental treatment									SEM***
	0.75 g AE*/kg	**0.50 g AE*/kg**	**0.25 g AE*/kg**	**0.75 g EE**/kg**	**0.50 g EE**/kg**	**0.25 g EE**/kg**	**Albio-vit**	**Bvit-extra**	**Negative control**	
Body weight, g										
14 d of age	216	211	227	200.9	206	213	202.1	213.7	200	9.4
28 d of age	886.7^{a}	814^{b}	721^{c}	615.5^{ef}	643^{e}	664^{de}	693^{cd}	707.5^{cd}	588^{f}	12.1
42 d of age	1859.1^{a}	1800^{b}	1684.7^{c}	1507^{e}	1545^{de}	1585^{d}	1661^{c}	1680^{c}	1101^{f}	16.7
56 d of age	2920^{a}	2850^{b}	2565.9^{d}	2466.7^{e}	2500^{e}	2600^{d}	2665^{c}	2660.6^{c}	1970^{f}	21.3
Daily weight gain, g/d										
1-14 d of age	12.00	11.64	12.79	10.92	11.29	11.79	11.01	11.84	10.86	0.18
15-28 d of age	47.91^{a}	43.07^{a}	35.29^{b}	29.61^{c}	31.21^{c}	32.21^{bc}	35.06^{b}	35.27^{b}	27.71^{c}	0.43
29-42 d of age	69.46^{a}	70.43^{a}	68.84^{a}	63.68^{c}	64.43^{bc}	65.79^{ab}	69.14^{ab}	69.46^{a}	36.64^{d}	0.61
43-56 d of age	75.78^{a}	75.00^{a}	62.94^{c}	68.55^{b}	68.21^{b}	72.50^{ab}	71.71^{ab}	70.04^{ab}	62.07^{c}	1.21
1-56 d of age	51.29^{a}	50.04^{ab}	44.96^{c}	43.19^{c}	43.79^{c}	45.57^{bc}	46.73^{abc}	46.65^{abc}	34.32^{d}	1.16
Daily feed intake, g/d										
1-14 d of age	14.64	14.71	14.84	14.56	14.79	14.70	14.64	14.62	14.57	0.29
15-28 d of age	45.50^{a}	44.86^{a}	44.31^{a}	41.88^{bc}	42.72^{abc}	43.25^{ab}	42.73^{abc}	43.61^{ab}	39.99^{c}	0.94
29-42 d of age	80.86^{a}	79.26^{ab}	75.64^{c}	71.08^{d}	72.13^{d}	74.13^{cd}	76.75^{bc}	75.09^{c}	63.41^{e}	1.31
43-56 d of age	128.91^{a}	126.79^{a}	120.46^{b}	115.18^{c}	117.09^{c}	121.13^{b}	121.6^{b}	121.69^{b}	99.43^{d}	2.55
1-56 d of age	67.48^{a}	66.40^{ab}	63.81^{bc}	60.68^{c}	61.68^{c}	63.30^{c}	63.93^{bc}	63.75^{bc}	54.35^{c}	1.78
Feed Conversion Ratio, g:g										
1-14 d of age	1.22	1.26	1.16	1.33	1.31	1.25	1.33	1.24	1.34	0.01
15-28 d of age	0.95^{d}	1.04^{cd}	1.26^{ab}	1.41^{ab}	1.37^{ab}	1.34^{ab}	1.22^{bc}	1.24^{b}	1.44^{a}	0.01
29-42 d of age	1.16^{b}	1.13^{b}	1.10^{b}	1.12^{b}	1.12^{b}	1.13^{b}	1.11^{b}	1.08^{b}	1.73^{a}	0.01
43-56 d of age	1.70	1.69	1.91	1.68	1.72	1.67	1.70	1.74	1.60	0.02
1-56 d of age	1.32^{b}	1.33^{b}	1.42^{ab}	1.40^{ab}	1.41^{ab}	1.39^{ab}	1.37^{b}	1.37^{b}	1.58^{a}	0.01

*Aqueous extract; **Ethanol extract; ***Standard error of mean; $^{a\text{-}f}$ Values in the same row not sharing a common superscript differ p<0.05

Table 5 shows the carcass yield of the broilers and their relative mean weights of organs as a percentage of live weight. In the current trial, no significant differences (p>0.05) were observed for the carcass yield and the internal organ weights of the broilers.

Table 5: Effects of the extracts on carcass yield and internal relative organ weight (%) of the broilers at day 56

Item	Experimental treatment									SEM***
	0.75 g AE*/kg	0.50 g AE*/kg	0.25 g AE*/kg	0.75 g EE**/kg	0.50 g EE**/kg	0.25 g EE**/kg	Albio-vit	Bvit-extra	Negative control	
Carcass (%)	94.25	92.13	90.91	94.59	94.67	93.59	93.75	95.00	94.91	0.44
Small intestine (%)	5.49	4.94	4.29	4.71	4.23	4.93	4.6	4.34	5.09	0.16
Liver (%)	3.14	2.24	2.14	3.43	3.46	4.48	3.35	3.04	3.64	0.11
Kidney (%)	0.06	0.09	0.07	0.06	0.06	0.06	0.06	0.05	0.03	0.02
Heart (%)	0.10	0.12	0.09	0.11	0.10	0.10	0.08	0.08	0.07	0.02

*Aqueous extract; **Ethanol extract; ***Standard error of mean

Blood protein

The blood protein concentrations of the broilers are presented in Figure 1. The groups fed aqueous leaf extract had significantly higher albumin values ($p < 0.05$) compared to the groups fed with ethanol extract. The albumin and globulin values obtained from the broilers fed aqueous leaf extracts were not significantly different ($p>0.05$) from the albumin and globulin values obtained in the groups fed Albiovit and Bvitetra (positive control groups). The maximum albumin value (4.265 ± 0.064 g/l) was recorded in broilers fed 0.25 g/kg aqueous leaf extract. Also, the highest globulin value (40.715 ± 0.163 g/l) was observed in the group fed 0.25 g/kg aqueous leaf extract. Conversely, the lowest albumin value (1.39 ± 0.071 g/l) and lowest globulin value (17.93 ± 0.806 g/l) were recorded in the negative control group. The negative control group had albumin and globulin values which were significantly different ($p < .05$) from the albumin and globulin values of the other groups.

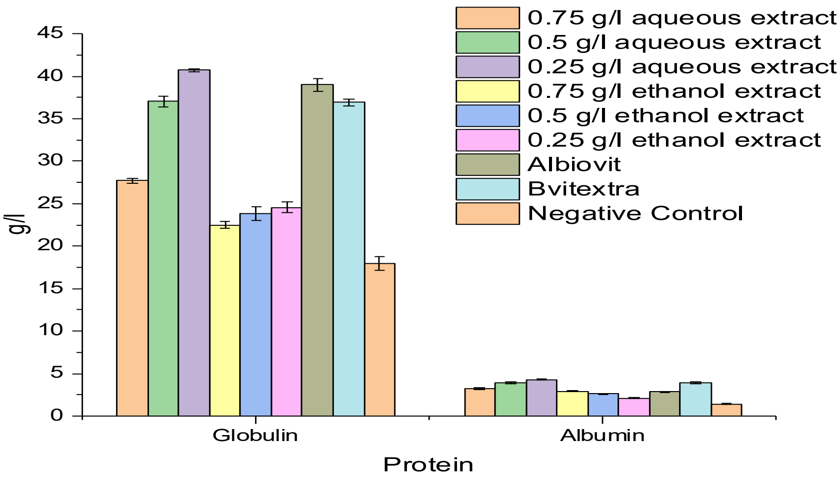

Figure 1: Effects of the aqueous and ethanol leaf extract on the blood protein concentrations of the broilers. Values are presented as mean ±SD

Discussion

This work set out to examine *J. secunda* as a possible alternative to antibiotics and other synthetic growth promoters in broiler production. Researchers have opined that a practical alternative to antibiotic growth promoters must exert a positive impact on feed conversion and the general growth of broilers (24, 25). In this current trial, the broilers' growth performance (Table 4) was significantly enhanced by the aqueous and ethanol leaf extracts of *J. secunda*. The body weight, feed intake, and feed conversion ratio of the broilers were all improved by the extracts. These marked effects of the extracts on the broilers' performance indices could be attributed to the many vitamins, minerals, flavonoids, and polyphenols they contain. The water-soluble vitamins and fat-solvable vitamins contained in the extracts (Table 1) meet the minimum dietary requirements for broilers (26, 27). According to Pal (28), vitamin A is essential for broiler production due to its ability to scavenge free radicals in the system, support growth, and promote eye function. In the same way, vitamin D is utilized in broiler production to maintain bone health by promoting gene expression in the small intestine that regulates intestinal calcium and phosphorus absorption, as well as promoting mineralization and calcium reabsorption in bones (29). The presence of these vitamins at permissible limits indicates that the leaf extracts can enhance the number of vitamins available to broilers and subsequently improve the growth performance of the broilers, making their contribution to the overall carcass yield, bone structure maintenance, and meat quality (28).

The inclusion of minerals in poultry diets is of great significance as it is implicated in their growth and development. Minerals aid in skeleton formation, function as enzyme cofactors, and are essential for maintaining osmotic balance in the poultry body (30). The macro and trace elements contained in the extracts (Table 2) are essential for broiler production. This finding is in agreement with the reports of other scientists for this plant (31, 32). The minerals contained in the leaf extracts suggest that supplementing broiler feed with the extracts increases the availability of the minerals for the broiler chickens. These essential minerals when deficient in the broiler diet can result in chronic metabolic disorders, affecting performance and carcass characteristics (32). Arsenic and nickel, both heavy metals, are within the maximum allowable limits of 30 ppm and 250 ppm in broiler production, respectively (33, 34). Arsenic is important in poultry feed as it aids in the biosynthesis of methionine and cysteine, while a nickel deficiency is thought to contribute to growth retardation, reduced immunity, shortening and thickening of leg bones, as well as joint swelling (35).

Additionally, flavonoids and polyphenols found in the leaf extracts (Table 3) played crucial roles as they contributed to the observed improvement in the broiler feed conversion ratio and body weight gain. This is due to the many healing effects of these phytochemicals. Onoja *et al*. (36) reported that the flavonoids and polyphenols contained in *J. secunda* were mostly responsible for the anti-inflammatory, antioxidant, and antinociceptive properties of this plant. In addition, flavonoids have been implicated in possible roles in preventing several chronic diseases associated with oxidant effects in poultry and other animals and enhancing the growth performance, blood composition, carcass composition, and morphology of the small intestine of broilers (37). Again, polyphenols are considered the primary contributors to antioxidant activity in *J. secunda* (14). Prihambodo *et al*. (37) reported that increasing flavonoid and polyphenol levels in broiler diets resulted in significant improvement in the growth performance of the broilers.

Similarly, the findings of this current trial revealed that neither the extracts nor the commercial growth promoters (Albiovit and Bvitextra) had any significant impact on the relative organ weight and the carcass yield of the broilers (Table 5). These findings are in line with the reports of other scientists who opined that feeding broilers with diets supplemented with phytogenic blends resulted in an improvement in broiler performance without any impact on carcass yield (38, 39, 40, 41, 42, 43).

Additionally, the increased serum globulin levels of the broilers (Figure 1) by the leaf extracts are consistent with the observations of Zhang *et al*. (44), who reported an improvement in the plasma globulin levels of broilers fed a dietary inclusion of phytogenic blends. The observed increase is an indicator of the ability of broiler chickens to administer phytogenic blends, such as the extracts of *J. Secunda*, to store excess protein even after the maximum protein accumulation capacity in the tissues of the broilers had been exceeded (45). Again, the significant increase in the albumin level of the broilers administered aqueous extract is in line with the report of Abd El-Hady *et al*. (46) who supported the claim that phytobiotics have positive effects on broiler serum albumin. The high levels of albumin observed in the serum of broiler chickens also indicated improved nutrient delivery and transportation in the system (45), thereby contributing to the observed growth performance of chickens.

Conclusion

This study investigated aqueous and ethanol leaf extracts of *J. secunda* as growth promoters in broiler production. The extracts were observed to promote broiler growth but had no significant effects on carcass characteristics and internal organ morphology. Additionally, the serum's biochemical parameters were elevated within normal limits to demonstrate that the extract was safe at certain concentrations. It is concluded from the study that *J. secunda* leaf extracts could serve as potential replacements for chemical growth promoters in poultry feed formulations. Nevertheless, the effects of the aqueous extract on the broilers were better than those observed for the ethanol extract. Therefore, the results of this study suggest that this plant can be exploited as an alternative to antibiotics used as growth promoters in broiler industries.

List of Abbreviations

J. secunda: Justicia secunda
BW: Body weight
DFI: Daily feed intake
DWG: Daily weight gain
FCR: Feed conversion ratio

Declarations

Ethical approval and consent to participate

All applicable international, national, and/or institutional guidelines for the care and use of animals as obtainable in Ebonyi State University, Abakaliki, Nigeria were followed. There was no special clearance and/or approval certificate obtained as of the time of this study.

Consent for publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution- Non-Commercial 4.0 license.

Availability of data and materials

The data and materials associated with this research will be made available by the corresponding author upon reasonable request.

Competing interests

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.

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Conceptualization: EE and AO; Material collation: EE; Methodology: EE and AO; Statistical analysis: SE and EE; Resources: EE and AO; Supervision: AO, EU, and SE; Writing – Original draft preparation: EE; Writing – Review and Editing: AO, SE, SN, EU, CU, KU, SU and CI. Approval of final draft: EE, AO, KU, SE, SN, EU, CI, CU, and SU.

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