

Comparative Evaluation of Selected Medicinal Plant Extracts on Health Biomarkers and Antimicrobial Activity in Broiler Chickens

Halimatu Sadiya Abdullahi*, Moses Zira Zaruwa, Ijeomah Ann Ukamaka, Chibuzo Carole Nweze

Department of Biochemistry. Nasarawa State University, Keffi. Nasarawa State, Nigeria.

Corresponding author*

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Abstract: This study presents a comparative evaluation of the phytochemical composition and health-promoting potential of seven medicinal plant extracts *Azadirachta indica*, *Curcuma longa*, *Allium sativum*, *Ocimum gratissimum*, *Vernonia amygdalina*, *Aloe barbadensis*, and *Moringa oleifera* on the health biomarkers of broiler chickens. By integrating phytochemical profiling, biochemical assessment, hematological indices, and antimicrobial testing, this research provides a multi-dimensional understanding of phytochemicals. Phytochemical screening confirmed the presence of phenols, tannins, alkaloids, flavonoids, saponins, and cardiac glycosides in varying concentrations. Among the extracts, *Ocimum gratissimum* and *Azadirachta indica* contained the highest flavonoid and saponin levels, respectively. *Vernonia amygdalina* and *Moringa oleifera* exhibited superior effects on liver and kidney biomarkers, enhancing total protein while reducing urea and creatinine levels. *Allium sativum* notably improved hematological parameters such as hemoglobin concentration, while *Azadirachta indica* increased total white blood cell counts. Antimicrobial screening demonstrated that *Moringa oleifera* and *Vernonia amygdalina* possessed the strongest inhibition against pathogenic bacteria, including *Salmonella spp.*, *E. coli*, and *Klebsiella spp.* These findings reveal the synergistic potential of medicinal plants as phytochemical feed additives to improve organ health, immunity, and microbial safety in poultry production. The comparative approach enhances understanding of plant specific effects and supports development of natural alternative to antibiotics in broiler chicken.

Key words: Phytochemicals, Broiler Chickens, Liver and Kidney function, Hematological Indices, Antimicrobial Activity, Antibiotic alternative.

I. Introduction

Medicinal plants remain the most abundant bioresources used for therapeutic purposes in both human and veterinary medicine. Their increasing application in livestock production, particularly as phytochemical feed additives, addresses the urgent need to reduce dependence on synthetic antibiotics that contribute to microbial resistance and residues in animal products. Numerous studies have explored individual plant species such as *Moringa oleifera* and *Azadirachta indica* for their growth-promoting and immunomodulatory properties. However, comparative assessments examining multiple plants simultaneously remain scarce. This study aims to fill that gap by conducting a comparative evaluation of seven medicinal plants commonly utilized in traditional African ethnoveterinary practice. By analyzing their phytochemical composition, biochemical impacts, hematological responses, and antimicrobial efficacy, this research provides integrated evidence for their collective and differential contributions to broiler chicken health.

II. Materials and Methods

Ethical Clearance: Ethical approval was obtained from the Ethical Committee, Nasarawa State University, Keffi.

Preparation of Plant Extract

Azadirachta indica, *Curcuma longa*, *Allium sativum*, *Ocimum gratissimum*, *Vernonia amygdalina*, *Aloe barbadensis*, *Moringa oleifera* fresh leaves were obtained from local farmers in Keffi, area of Nasarawa state, Nigeria. The whole plants were washed under running tap water, shade dried at room temperature, and powdered. The powdered plant samples (500g/150ml) of solvent was added and kept for 3 days. The extract was filtered using Whatman No.1 filter paper and the supernatant was collected. The residue was again extracted two times (with 3 days of interval for each extraction) and supernatants were collected. The supernatants were evaporated in water bath $28 \pm 1^\circ\text{C}$ until the volume was reduced to 150 ml. Extract of the whole plant powder was prepared and stored in air tight bottles for subsequent analysis.

Phytochemical Analysis

The phytochemical analysis were carried out on the plant extracts using the standard procedures. The presence or absence of a particular phytochemical compound involved the addition of appropriate standard chemicals/reagents in appropriate sequence to the plant extract. The following classes of phytochemicals were screened for: phenols, tannins, alkaloids, flavonoids, cardiac glycosides, saponins, terpenoids, steroids, glycosides

Uv- Vis Spectrum Analysis

The extract was centrifuged at 3000rpm for 10min and filtered through Whatmann No.1 filter paper. The sample was diluted to 1:10 with the same solvent. The extract was scanned at wave length ranging from 200 to 1100 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. The peak values of the UV-VIS were recorded.

Experimental Design

A total of 32 healthy day old broiler chicks were purchased from a reputable veterinary shop in Keffi, Nasarawa State and housed at the animal house of the Department of Biochemistry, Nasarawa State University, Keffi. The birds were acclimatized for two week before grouping and two weeks after the grouping the treatment commenced and lasts for another two weeks. The broiler chicken were grouped as follows;

Group 1 (control): Received 2L of water + 50g of normal feed

Group 2: Received of 300mg/kg bw *Vernonia amygdalina* extract in 2L of water + 50g of normal feed

Group 3: Received 200mg/kg bw of *Azadirachta indica* extract in 2L of water + 50g of normal feed.

Group 4: Received 330mg/kg of *Ocimum gratissimum* extract in 2L of water + 50g of normal feed.

Group 5: Received 400mg/kg of *Allium sativum* extract in 2L of water + 50g of normal feed

Group 6: Received 220mg/kg bw of *Curcuma longa* extract in 2L of water + 50g of normal feed.

Group 7: Received 500mg/kg bw of *Aloe barbadensis* extract in 2L of water + 50g of normal feed.

Group 8: Received 500mg/kg bw of *Moringa oleifera* extract in 2L of water + 50g of normal feed.

Blood Sample Collection

The animals were sacrificed in accordance with the guidelines of the European Convention for the Protection of Vertebrate Animals and other Scientific Purposes ETS-123 (European Treaty Series, 2005). Whole blood was collected using sterile syringe and needle. The blood sample of each chicken was collected into respective capped tubes and was centrifuged at 1000 rpm for 10 minutes using a bench top centrifuge to separate cells from serum and stored in the refrigerator for further analysis.

Biochemical Analysis

The serum obtained was used to analyse for Liver function parameter (Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), Protein following standard methods of Reitman and Frankel (1957) and kidney function parameters (Sodium ion (Na⁺), Potassium ion (K⁺), Urea and Creatinine) following standard methods of Bartels and Bohmer (1972) with modification as outlined in Randox kit.

Haematologica Analysis

Whole blood samples were used for haemological parameters (white Blood cell (WBC), Red blood cell (RBC), Pack cell volume (PCV), Hemoglobin (Hb), mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) and white cell differentials; Neutrophil (N), Leukocyte (L), Mesophil (M), Eosnophil (E))and Basophil (B) Following standard methods of Williams *et al.*, (1972).

Microbiological Analysis

Plant extracts were inoculated in agar plates, allowing the bioactive components to diffuse into the agar and interact with the microorganisms. The growth of the microorganisms is inhibited by the various plant extract creating a clear zone around the disk. The size of the zone of inhibition is measured, indicating the bioactive components in the sample.

Statistical Analysis

The data obtained were analyzed using one-way ANOVA in IBM SPSS version 23.0 to get the means and standard deviations (n=4). Further test for levels of significance was considered statistically at a level of p<0.05 (Duncan, 1957).

III. Results

Table 1: Qualitative phytochemical screening of Different Plant Extracts

Metabolites	<i>C. longa</i>	<i>V. amygdalina</i>	<i>M. oleifera</i>	<i>O. gratissimum</i>	<i>A. indica</i>	<i>A. barbadensis</i>	<i>A. sativum</i>
Phenols	+	+	+	+	++	+	+
Tannins	+	+	+	+	+	+	+

Alkaloids	+++	+	+	+	+	+	+
Flavonoids	++	+	+	+	+++	+	+
Cardiac Glycosides	+	+	+	+	++	-	+
Saponins	++	+	+	+	+++	+	+
Terpenoids	-	+	+	+	+++	-	+
Steroids	++	+	+	-	+	-	-
Glycosides	-	-	+	-	-	+	+

Key: + = Present; ++ = Moderate; +++ = Abundant; - = Absent.

Highest phytochemical abundance observed in *A. indica* (saponins, terpenoids) and *C. longa* (alkaloids).

Table 2. Quantitative Phytochemical Concentrations (g/mg) of Different Plant Extracts

Metabolite	<i>C. longa</i>	<i>V. amygdalina</i>	<i>M. oleifera</i>	<i>O. gratissimum</i>	<i>A. indica</i>	<i>A. barbadensis</i>	<i>A. sativum</i>
Phenols	8.60 ± 0.51	253.56 ± 2.37	171.33 ± 3.06	531.44 ± 4.57	43.79 ± 1.72	34.94 ± 0.52	39.10 ± 0.45
Tannins	11.29 ± 0.92	76.89 ± 1.44	47.18 ± 1.54	175.43 ± 4.49	10.52 ± 1.11	25.49 ± 1.43	24.24 ± 0.54
Alkaloids	75.44 ± 0.52	76.86 ± 2.68	98.69 ± 0.42	86.88 ± 2.06	28.84 ± 0.43	32.82 ± 1.07	29.74 ± 0.73
Flavonoids	40.74 ± 0.63	232.85 ± 2.05	30.67 ± 2.52	298.03 ± 2.82	199.46 ± 1.01	17.97 ± 0.84	73.64 ± 1.10
Saponins	47.19 ± 2.00	282.96 ± 2.34	82.33 ± 1.25	161.54 ± 2.26	677.95 ± 4.84	11.68 ± 1.41	21.09 ± 0.70
Terpenoids	–	21.03 ± 1.49	40.89 ± 0.77	23.47 ± 1.27	161.75 ± 1.54	–	15.80 ± 0.69

Highlight: *O. gratissimum* exhibited the highest total phenolic and flavonoid content, while *A. indica* contained the highest saponin and terpenoid concentrations.

Table 3. Liver Function Parameters in Broiler Chickens Administered with Different Plant Extracts

Group	Total Protein (g/L)	ALP (UI/L)	ALT (UI/L)	AST (UI/L)	Interpretation
Control	56.00 ± 10.47	310.50 ± 27.99	15.60 ± 6.08	169.30 ± 17.35	–
<i>V. amygdalina</i>	75.38 ± 11.90	269.38 ± 29.89	12.78 ± 0.47	150.54 ± 8.56	↑ protein, hepatoprotective
<i>A. indica</i>	63.18 ± 4.65	220.00 ± 6.16	13.95 ± 2.57	155.34 ± 12.17	↓ ALP, hepatoprotective
<i>O. gratissimum</i>	69.50 ± 8.57	84.30 ± 3.35	12.36 ± 5.22	131.10 ± 0.82	Strong hepatoprotection
<i>A. sativum</i>	79.68 ± 6.55	315.75 ± 4.35	11.83 ± 3.54	149.08 ± 3.72	↑ protein synthesis
<i>C. longa</i>	55.38 ± 8.99	250.40 ± 37.76	12.33 ± 2.05	160.83 ± 1.72	Mild effect
<i>A. barbadensis</i>	58.65 ± 6.60	84.50 ± 4.50	16.38 ± 1.64	133.42 ± 0.47	Mild improvement
<i>M. oleifera</i>	59.38 ± 8.44	237.70 ± 15.93	16.58 ± 4.64	171.03 ± 6.16	Moderate effect

Best performers: *V. amygdalina*, *A. indica*, and *O. gratissimum* showed strongest hepatoprotective effects.

Table 4. Kidney Function Parameters in broiler chickens Administered with Different Plant Extracts

Group	Na ⁺ (mmol/L)	K ⁺ (mmol/L)	Urea (mmol/L)	Creatinine (mmol/L)	Interpretation
Control	167.20 ± 3.11	3.75 ± 0.35	7.30 ± 0.57	69.40 ± 5.51	–
<i>V. amygdalina</i>	139.05 ± 0.82	3.59 ± 0.09	3.11 ± 0.09	42.68 ± 5.19	Strong nephroprotection
<i>A. indica</i>	137.75 ± 1.21	3.88 ± 0.22	3.33 ± 0.25	59.68 ± 1.81	Improved kidney function
<i>M. oleifera</i>	166.28 ± 2.58	3.63 ± 0.53	2.20 ± 0.08	49.83 ± 4.26	Best renal improvement
<i>C. longa</i> , <i>O. gratissimum</i> , <i>A. sativum</i> , <i>A. barbadensis</i>	–	–	–	–	Mild-to-moderate effects

Key: Best performers-*M. oleifera* > *V. amygdalina* > *A. indica* for renal protection.

Table 5. Hematological Parameters of Broiler Chickens Administered with Different Plant Extracts

Parameter	Control	<i>V. amygdalina</i>	<i>A. indica</i>	<i>A. sativum</i>	<i>M. oleifera</i>	Key Observation
WBC (×10⁹/L)	4.10 ± 0.42	6.56 ± 1.41	26.63 ± 9.35	6.58 ± 0.57	13.13 ± 6.46	<i>A. indica</i> markedly increased WBC, indicating enhanced immune response
Hb (g/dl)	11.55 ± 0.49	10.99 ± 2.33	14.68 ± 1.25	16.93 ± 0.97	13.93 ± 0.31	<i>A. sativum</i> showed highest hemoglobin value, enhancing oxygen transport
RBC (×10¹²/L)	4.40 ± 0.14	4.73 ± 0.13	5.58 ± 0.62	6.35 ± 0.21	5.43 ± 0.46	<i>A. sativum</i> stimulated erythropoiesis
PCV (%)	37.00 ± 1.41	33.75 ± 6.24	47.25 ± 1.71	31.25 ± 2.22	42.25 ± 1.71	<i>A. indica</i> improved packed cell volume
MCV (fL)	83.50 ± 0.71	124.00 ± 10.13	233.75 ± 15.19	114.73 ± 5.59	69.00 ± 2.94	<i>A. indica</i> improved erythrocyte morphology
MCH (pg)	26.45 ± 0.21	41.14 ± 3.68	43.50 ± 1.29	36.63 ± 1.60	22.75 ± 0.96	<i>A. indica</i> and <i>A. sativum</i> enhanced hemoglobin content
MCHC (g/dl)	31.35 ± 0.07	33.26 ± 0.05	33.00 ± 0.61	33.20 ± 0.00	33.00 ± 0.82	Stable across groups, slight improvement noted
Neutrophils (%)	5.00 ± 1.41	11.93 ± 3.84	33.25 ± 2.22	32.25 ± 3.40	11.25 ± 1.71	<i>A. indica</i> induced strong immune activation
Lymphocytes (%)	85.00 ± 2.83	67.25 ± 1.71	60.75 ± 1.71	62.00 ± 1.63	81.75 ± 2.22	Lymphocyte balance maintained
Eosinophils (%)	2.50 ± 0.71	2.75 ± 0.96	1.50 ± 0.58	2.75 ± 0.50	2.50 ± 0.58	No significant variation

Interpretation: *Allium sativum* enhanced erythropoietic parameters (Hb, RBC), while *A. indica* improved immune and hematological indices (WBC, neutrophils, PCV).

Table 6. Antimicrobial Activity of Plant Extracts (Zone of Inhibition)

Bacterial Isolate	<i>V. amygdalina</i>	<i>A. indica</i>	<i>O. gratissimum</i>	<i>C. longa</i>	<i>M. oleifera</i>	Interpretation
<i>Salmonella spp.</i>	26mm	26mm	25mm	24mm	25mm	Broad-spectrum inhibition observed across extracts

<i>E. coli</i>	24mm	26mm	27mm	26mm	26mm	High activity against Gram-negative bacteria
<i>Klebsiella spp.</i>	24mm	0mm	24mm	26mm	27mm	Strongest inhibition by <i>M. oleifera</i>
<i>Pseudomonas spp.</i>	27mm	26mm	26mm	24mm	22mm	Effective control by <i>V. amygdalina</i> and <i>A. indica</i>
<i>Staphylococcus aureus</i>	27mm	26mm	0mm	25mm	27mm	Potent Gram-positive inhibition by <i>M. oleifera</i> and <i>V. amygdalina</i>

Key: *Moringa oleifera* and *Vernonia amygdalina* demonstrated the broadest antimicrobial spectra, effectively inhibiting both Gram-positive and Gram-negative bacteria, followed by *A. indica*.

V. Discussion

The comparative analysis of the seven plant extracts revealed that each species contributed differently to the physiological and biochemical well-being of broiler chickens. The high levels of flavonoids and phenolic compounds (Table1) observed in *Ocimum gratissimum* and *Azadirachta indica* corroborate findings from Zeng *et al.* (2015) and Perez-Gregorio *et al.* (2021), who reported that flavonoid-rich phytobiotics enhance liver detoxification and oxidative stability in poultry.

Vernonia amygdalina and *Moringa oleifera* (Table 3 and 4) demonstrated marked improvements in liver and kidney function, consistent with studies from Agbetuyi and Oloruntola (2020) and Mohammed *et al.* (2021), suggesting their hepatoprotective and nephroprotective properties.

Vernonia amygdalina and *Moringa oleifera* demonstrated marked improvements in liver and kidney function, consistent with studies from Agbetuyi and Oloruntola (2020) and Mohammed *et al.* (2021), suggesting their hepatoprotective and nephroprotective properties. The increase in serum protein levels indicates improved protein metabolism and nutrient utilization, critical for growth performance and immune competence.

The hematological results (Table 5) also align with previous reports that *Allium sativum* enhances erythropoiesis and immune cell activation due to its organosulfur compounds. Elevated WBC counts following *Azadirachta indica* supplementation suggest enhanced immune readiness, consistent with global reports on neem's immunomodulatory capacity (Khater *et al.*, 2020).

The broad-spectrum antimicrobial activity (Table 8), particularly from *Moringa oleifera* and *Vernonia amygdalina*, supports their traditional use as natural antimicrobials. Similar inhibition patterns have been observed in Asian and European poultry trials, reinforcing the global potential of these plants as safe antibiotic alternatives. This study therefore provides novel comparative insights, demonstrating that the simultaneous assessment of multiple phytogetic plants offers a more holistic understanding of functional diversity.

VI. Conclusions and Future Perspectives

The findings of this study confirm that medicinal plants such as *Moringa oleifera*, *Vernonia amygdalina*, *Azadirachta indica*, and *Allium sativum* provide complementary health benefits when used as phytogetic feed additives in broiler chickens. They enhance liver and kidney function, improve hematological profiles, and exhibit potent antimicrobial activity. The comparative approach strengthens the understanding of their differential effects and highlights their collective potential to promote poultry production.

Future studies should focus on larger sample sizes, longer trial durations, and inclusion of growth performance, carcass quality, and economic analyses to establish commercial feasibility. Integrating metabolomic and genomic tools could further elucidate the molecular mechanisms underlying these beneficial effect.

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