SVU- International Journal of Veterinary Sciences, 7(3): 51-63 , 2024
Print ISSN: 2535-1826 Online ISSN: 2535-1877

Research Article Open Access

In-vitro and *in-vivo* antibacterial and therapeutic activity of methanol extract of whole fruit of *Lagenaria breviflora* against *Salmonella* species in broilers

Tolulope Ademola Olakojo1*, Olayinka Ayotunde Oridupa1, Adebowale Bernard Saba1

[1]*Department of Veterinary Pharmacology and Toxicology, University of Ibadan, Ibadan, Nigeria.*

Abstract

Salmonellosis, antimicrobial drug residues, and multi-drug resistance are major challenges accounting for a huge loss within the poultry investment scheme. *In-vivo* and *in-vitro* antibacterial prospect, utilizing the methanol extract of *Lagenaria breviflora* (LB), was applied as an alternative therapy for salmonellosis in broilers. Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC) were determined and showed an improved inhibition performance. The *in-vivo* assay was performed on 60 broilers in various treatment groups from treatment 1-6 with 10 times repetition. Treatment 1 was uninfected and untreated. Treatment 2-6 were infected with *Salmonella* species *per os.* After 6 days of incubation, treatment 3-6 received treatment with LB extract (200 mg/kg, 400 mg/kg, 800 mg/kg) or ciprofloxacin (20 mg/kg), respectively, for 5 days. The broilers in treatment 2, in contrast, did not receive any therapy. Fecal samples from Treatment 3-5 were significant ($p \leq 0.05$), indicating lower bacterial counts compared to Treatment 2, and Treatment 1. Elevated PCV, and WBC were lower in Treatment 3-5. These latter groups had hemogram values comparable to infected chickens treated with ciprofloxacin and healthy control chickens. Histopathology showed reversed liver inflammation, and an amelioration of degenerated nephrons, in extract-treated broilers, accompanied by a better control of diarrhoea and dehydration. This study, therefore, established the antibacterial potential of *L. breviflora* against *Salmonella* species in broilers. Diarrhea and dehydration associated with salmonellosis were also reversed in extract-treated broilers.

Keywords: *Lagenaria breviflora*; Salmonellosis; Broiler chickens; Diarrhea

DOI: 10.21608/svu.2024.302808.1329 Received: 2024-07-09 Accepted: 2024-08-29 Published: 2024-08-31

*Corresponding Author: Tolulope Ademola Olakojo
E-mail: mailintolulope@gmail.com/ ta.olakojo@ui.edu.ng.

Citation: In-vitro and in-vivo antibacterial and therapeutic activity of methanol extract of whole fruit of Lagenaria breviflora against Salmonella species in broilers. SVU-IJVS 2024, 7(3): 51-63.

Competing interest: The authors have declared that no competing interest exists.

Introduction

Antibiotic inclusion in poultry ration to enhance feed conversion, growth and prevent mortality from clinical diseases has been common practice (Ab El-Hack et al., 2022). The Food and Drug Administration (FDA), World Health Organization (WHO), and European Medicines Agency (EMEA) recommend avoiding synthetic drugs due to residues in animal products (Van Hoogevest and Wendel, 2014). Ethno-botanicals are alternatives employed instead of synthetic drugs. Biochemical properties of some plant essential oils make them suitable alternatives to compounded manmade drugs (Puvača et al., 2013; Aćimović et al., 2020) to ensure food safety, less dependence on synthetic drugs, and antimicrobial resistance mitigation. Many plants are rich in phytocompounds which are frequently inexpensive, tolerated by consumers, and generally eco-friendly (Durmic and Blache, 2012; Kostadinović et al., 2015). A few of these natural plants have been used in folklore medicine to manage diarrhea, flu, skin diseases, and bacterial infections including typhoid fever caused by *Salmonella* species (Roger et al., 2015).

Salmonella gallinarum, responsible for fowl typhoid in poultry production, has been incriminated in several incidences of high mortality. The disease is endemic to Nigeria with a high prevalence rate, and therapeutic efforts with existing drugs are scuttled by the daily inclusion of antibiotics and chemicals in feed. Natural or synthetic antibiotics are used for prophylactic and curative purposes to inhibit or kill bacteria reducing the challenge of antibiotic resistance (Forgetta et al., 2012) and the antibiotic remnant in food as well as the environment (Carvalho and Santos, 2016; Gonzalez Ronquillo and Angeles Hernandez, 2017). Consequently, increased demand for therapeutic options to control infectious diseases while constraining the widespread of resistant bacteria arises yet being mindful of keeping antibiotics as a helpful tool.

Among several ethnobotanical options with promising compounds as antibiotic and anticoccidial effects is *Lagenaria breviflora* fruit, popularly known as 'Christmas Melon' and '*Tagiri*' by the Yoruba tribe. This fruit is primarily cultivated in African countries, particularly those with warm climates, such as Nigeria, Ghana, and Kenya. However, its cultivation is not limited to Africa alone (Dhillon et al., 2016). *L. breviflora* fruit is known to possess a wide array of benefits in terms of nutritional value additions (vitamins A and C), (Saed et al., 2022) as well as finding usefulness in medicine to manage inflammation, respiratory problems, digestive and viral infections (Adeyemi et al., 2017). This is plausible from earlier studies that reported the broad-spectrum activity against gram-positive and negative bacteria (Tomori et al., 2007). Therefore, this study seeks to explore *in vitro* and *in vivo* activities of *L. breviflora* against *Salmonella* species in broiler chickens including, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), bacterial count, hematology and serum biochemical indices as well as histopathological findings associated with the effect of the extract in the liver and kidney. This study equally seeks to explore alternative drug options available for use in poultry production, as a means to reduce the rising cost of production.

Materials and Methods

Preparation of Extracts and Salmonella Isolate

Lagenaria breviflora fruits were harvested from Bode-Igbo, Ido LGA, Ibadan. The identification of *L. breviflora* fruit was performed at the Herbarium of the Department of Botany, Faculty of Sciences, University of Ibadan

The dried, blended whole fruit sample (900g) was macerated in methanol (7L). After 72 hours of continuous extraction (Oridupa et al., 2018), the mixture was filtered (Whatman-filter-paper-10µm). A rotary evaporator (Heidolph laborota 400 efficient, Germany, Model 517-01002-002), set at 40^0C was

used to concentrate the filtrate, while moisture was removed in a vacuum oven (40^{0}C) at a pressure of 700mmHg. The weight of the crude extract was 155g.

Pure *Salmonella* (reference strain: 35664) was provided by the Department of Veterinary Public Health & Preventive Medicine Laboratory, University of Ibadan, Ibadan for this study.

In-Vitro Antibacterial Assays

Antibiotic Sensitivity Test

Diluted test isolate (*Salmonella)* was swabbed on Mueller Hinton agar plates. A sterilized 8mm cork borer was used to birth six wells on the agar plate. Each well was filled with 0.1ml of extract solutions (50 µg/ml , 100 µg/ml , 200 µg/ml , 400 µg/ml , 800 µg/ml) including ciprofloxacin (10 µg/ml). Leaving the plates for 1hr at room temperature, a 24-hour incubation (37 ^{0}C) followed through. A meter rule was used to measure the zones of inhibition (CLSI, 2018).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The broth microdilution method in 96-well plates was used to carry out MIC. The plant extract was dissolved in double-strength Tryptone soya Broth to obtain 400µl solution (Kowalska-Krochmal and Dudek-Wicher, 2021). This was diluted serially in a sterile 96-well plate to obtain concentration ranges of 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, and 3.125µg/ml. The wells were each inoculated with 10µl *Salmonella* broth (1.02 x 10^{8}CFU) and incubated (37 ^{0}C) for 24 hours. The concentrations without growth or turbidity 24 hours post-incubation were taken as MIC.

To each well, P-iodonitrotetrazolium violet (p-INT) solution (10 µl; 0.02%) was added and incubated (37 ^{0}C) for 30min. Bacterial/microbial growth was indicated by color change (yellow to pinkish red). The most negligible concentration, which showed no growth or color change, was considered MBC. The color changes were usually due to bacterial growth in the presence of the extract.

Experimental Animals

Sixty (60) broiler (Arbor acre) day-old chickens were acclimatized and brooded at Bora Farms, Institute of Agricultural Research and Training (IAR&T), Ibadan.

The chickens were divided into 6 equal treatment groups (TRT1-TRT6), at a day old. TRT1 being the control and TRT2; being infected-untreated. TRT3-TRT5 were infected but treated with 200mg/kg, 400mg/kg, or 800mg/kg body weight *L. breviflora* methanol extract, respectively, while TRT6 was infected and treated with ciprofloxacin (20mg/kg). Chicks were inoculated with 1.02 x 10^{8}CFU *Salmonella* isolate on day 14 (Audisio and 2002), and a definitive diagnosis was made on day 19 (5 days post-inoculation) by fecal culture on SSA following a drop in appetence and with diarhea, been the most visible clinical sign. Treatment commenced on day 20 (6th day post-inoculation) for the next 5 days following Lorke's method (Lorke, 1983) and the guidelines of OECD (2001).

Sample Collection

Fecal samples for bacteria culture were obtained randomly on days 0, 5, and 10 post-inoculation from all groups for bacteriology (CFU/ml on SSA), as described by Ruiz et al. (1996). Blood samples were collected on day10 post-inoculation (last day of treatment) for hematology (PCV, Hb, RBC, MCHC, MCV, MCH, WBC, Neutrophils, Eosinophils, Lymphocytes, and platelet) and serum chemistry (ALT, AST, ALP, Total-Protein, Albumin, Globulin, Total-Cholesterol, Triglyceride, Urea, Creatinine, Total-Bilirubin, Conjugated-Bilirubin, Glucose and Electrolytes) as described by Saba et al. (2010). Afterward, the chickens were humanely sacrificed while organ samples (liver and kidney) were collected and preserved in 10% formaldehyde. They were dehydrated, embedded in paraffin blocks, and prepared for histopathology.

Ethical Approval

The experimental design was subjected to review and approval by the board of the University of Ibadan Animal Care and Use Review Committee, with protocol number as stated herein (NHREC/UIACUREC/05/12/2022A).

Data Analysis

All data collected were subjected to descriptive statistics and expressed as mean±SD. One-way analysis of variance (ANOVA) with Turkey's post-hoc test was performed using Graph Pad Prism version 7.10. Values of $p \leq 0.05$ were considered significant.

Results

Antibiotic Sensitivity Test

In-vitro antibacterial assay of *Lagenaria breviflora* methanol extract against *Salmonella* species showed a concentration-dependent increase in the zones of inhibition (16.03±0.09 mm - 28.17±0.54 mm), while ciprofloxacin had significantly ($p<0.05$) larger zones of inhibition (45.4±1.02mm) (Figure1). MIC and MBC of the methanol extract of *L. breviflora* were also evaluated to be 100 µg/ml 200 µg/ml,

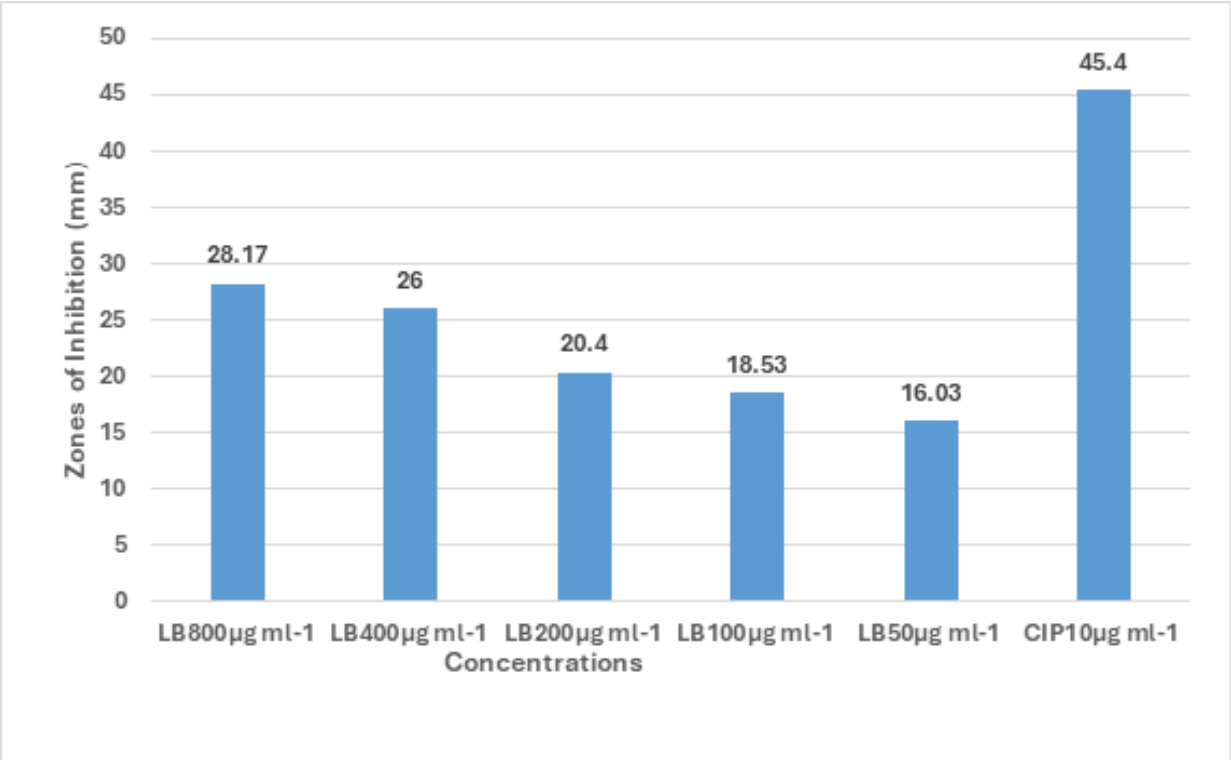

Figure 1: In vitro antibacterial effect of Lagenaria breviflora methanol extract against Salmonella species (Antibiotic Sensitivity Test)

Table 1: Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) in mg/ml of *L. breviflora* against *Salmonella* species

(Concentration (µg/ml	MIC	MBC
L. brev 3.725 µg/ml	-	-
L. brev 6.25 µg/ml	-	-
L. brev 12.5 µg/ml	-	-
L. brev 25 µg/ml	-	-
L. brev 50 µg/ml	-	-
L. brev 100 µg/ml	+	+
L. brev 200 µg/ml	+++	++

L. brev - L. breviflora extract, MIC – Minimum inhibitory concentration, MBC – Minimum

respectively (Table 1).

In-vivo Antibacterial Study

Bacteriology

Colony forming unit count per ml (CFU/ml) of *Salmonella spp.* within infected chickens was of high significance (p≤0.05) by day 5 compared to day 0 and healthy controls on day 5. A significant decline was observed in CFU/ml by day 10 post-inoculation in infected-treated chickens of treatment groups 3 (2.37±0.12), 4 (1.60±0.29), 5 (1.73±0.23) and 6 (2.73±0.58). The CFU/ml of infected-treated chickens that received the plant extract (treatment 3, 4 & 5) compared favorably well with that of chickens that received ciprofloxacin (2.73±0.58). However, a significant increase of CFU was observed for infected-untreated chickens (16.73±1.75) through the 10-day course of the experiment (Table 2).

Table 2: Colony forming units (CFU/ml) of *Salmonella sp* in chicken feces experimentally infected and treated with methanol extract of *L. breviflora* whole fruit. All values are expressed in 10^8 x CFU ml^{-1}

TRT	Treatment Groups	DAY 0 Post-Inoculation	DAY 5 Post-Inoculation	DAY 10 Post-Inoculation
TRT1	Control	2.03 ± 0.38	1.53 ± 0.18	2.00 ± 0.26
TRT2	Infected-Untreated	2.00 ± 0.15	6.30 ± 0.45	16.73 ± 1.75
TRT3	200 mg/kg LB + Salmonella	1.9 ± 0.17	6.27 ± 0.68	2.37 ± 0.12[b]
TRT4	400 mg/kg LB + Salmonella	1.6 ± 0.15	8.07 ± 0.69[a]	1.60 ± 0.29[b]
TRT5	800 mg/kg LB + Salmonella	1.23 ± 0.09	6.23 ± 0.39[a]	1.73 ± 0.23[b]
TRT6	20 mg/kg Cipro + Salmonella	1.9 ± 0.12	10.70 ± 1.22[a]	2.73 ± 0.58[b]

Mean±standard deviation; Values with superscript ([a]) are statistically significant ($p<0.05$) compared to control. Values with superscript ([b]) are statistically significant ($p<0.05$) compared to infected-untreated.

Hematology

Chickens infected and treated with *L. breviflora* had PCV values of 26.00±0.58, 29.00±1.16 and 28.33±3.21%, which were statistically unchanged compared to PCV of healthy chickens (29.33±0.88%). These were significantly lower compared to infected untreated chickens (34.00±2.31%) and infected chickens treated with ciprofloxacin (23.67±0.88%). Red blood cells and hemoglobin concentration of untreated-infected chickens were also significantly increased compared to infected-treated chickens with either extract or ciprofloxacin. Also, mean MCV and MCH values of infected-treated chickens with *L. breviflora* extracts were of notable significance (p≤ 0.05), and higher compared to infected-untreated chickens, while MCHC values were statistically unchanged (Table 3).

Leucogram showed significantly (p≤ 0.05) higher WBC (3.61±0.59), neutrophils (2.31±0.26), and lymphocytes count (1.18±0.52) for infected-untreated chickens compared to healthy controls and infected chickens treated with extract or ciprofloxacin. Platelet counts in infected chickens treated with extract or ciprofloxacin were statistically unchanged compared to control healthy chickens while infected-untreated chickens had significantly higher platelet count (Table 4).

T[ab]le 3: Red blood cell indices of chickens (healthy, experimentally infected with *Salmonella sp* either untreated or treated with methanol extract of *Lagenaria breviflora* whole fruit or ciprofloxacin)

	Treatment Group	PCV (%)	RBC Cells/mm^3 (x10^6)	Hb (g/dL)	MCV (fl)	MCH (pg)	MCHC (%)
TRT1	Control	29.33 ± 0.88	3.59 ± 0.31	0.94 ± 0.02	82.57 ± 7.76	2.65 ± 0.29	3.21 ± 0.05
TRT2	Infected-Untreated	34.00 ± 2.31	6.32 ± 0.85	1.12 ± 0.05	51.74 ± 4.91	1.70 ± 0.16	3.29 ± 0.15
TRT3	200 mg/kg LB + Salmonella	26.00 ± 0.58	2.43 ± 0.19^b	0.84 ± 0.03^b	101.30 ± 9.84^b	3.09 ± 0.43^b	3.05 ± 0.18
TRT4	400 mg/kg LB + Salmonella	29.00 ± 1.16	2.51 ± 0.26^b	0.94 ± 0.14	101.20 ± 9.59^b	3.76 ± 0.53^{ab}	3.78 ± 0.83
TRT5	800 mg/kg LB + Salmonella	28.33 ± 3.21	2.58 ± 0.54^b	0.77 ± 0.09^b	120.10 ± 44.78^b	3.07 ± 0.49^b	2.77 ± 0.84
TRT6	20 mg/kg Cipro + Salmonella	23.67 ± 0.88^b	2.82 ± 0.38^b	0.74 ± 0.02^b	92.72 ± 4.78	2.72 ± 0.56	2.93 ± 0.54

Mean±standard deviation; Values with superscript (^{a}) are statistically significant ($p<0.05$) compared to control. Values with superscript (^{b}) are statistically significant ($p<0.05$) compared to infected-untreated

Table 4: White blood cell indices of chickens (healthy, experimentally infected with Salmonella sp either untreated or treated with methanol extract of Lagenaria breviflora whole fruit or ciprofloxacin)

	TRT	WBC ($x10^3$ Cells mm^{-3})	NEUT Cells/mm^3 ($x10^3$)	LYMP Cells/mm^3 ($x10^3$)	EOS Cells/mm^3 ($x10^3$)	MON Cells/mm^3 ($x10^3$)	BAS Cells/mm^3 ($x10^3$)	PLT Cells/mm^3 ($x10^5$)
TRT1	Control	1.82 ± 0.53	1.07 ± 0.30	0.72 ± 0.25	0	0.02 ± 0.03	0	1.29 ± 0.10
TRT2	Infected-Untreated	3.61 ± 0.59	2.31 ± 0.26	1.18 ± 0.52	0.01 ± 0.02	0	0.01 ± 0.02	2.13 ± 0.10
TRT3	200 mg/kg LB + Salmonella	0.95 ± 0.05[b]	0.60 ± 0.10[b]	0.34 ± 0.05[b]	0	0.003 ± 0.006	0	1.33 ± 0.17
TRT4	400 mg/kg LB + Salmonella	0.79 ± 0.15[ab]	0.47 ± 0.09[ab]	0.30 ± 0.09[b]	0.002 ± 0.003	0.01 ± 0.01	0.003 ± 0.006	1.01 ± 0.31[b]
TRT5	800 mg/kg LB + Salmonella	1.02 ± 0.09[b]	0.68 ± 0.12[b]	0.33 ± 0.03[b]	0	0.01 ± 0.01	0	1.31 ± 0.51
TRT6	20 mg/kg Cipro + Salmonella	0.59 ± 1.32[b]	0.65 ± 0.09[b]	0.29 ± 0.06[b]	0.003 ± 0.005	0.04 ± 0.06	0	1.01 ± 0.16[b]

Mean±standard deviation; Values with superscript ([a]) are statistically significant ($p<0.05$) compared to control. Values with superscript ([b]) are statistically significant ($p<0.05$) compared to infected-untreated.

Serum Biochemistry

Total proteins and their constituent fractions were statistically unchanged for all groups while liver enzymes were marginally elevated in all infected chickens compared with healthy controls. Total bilirubin values in infected chickens treated with 400 and 800mg/kg were lower than in infected-untreated chickens but comparable to healthy controls (Table 5). Also, urea and creatinine values were lower in extract-treated compared to infected-untreated chickens, but comparable to healthy controls. The lipid profile of all infected chickens showed lower TC, TG, HDL, and LDL compared to the control. A further decline was observed in infected-untreated chickens compared to infected chickens treated with ciprofloxacin (Table 6).

Histopathology

Histopathology of the liver harvested revealed a moderate portal congestion, with mild periportal hydropic degeneration of hepatocytes observed in the infected-untreated group. However, this was was reversed in the extract-treated groups of 200 and 400mg/kg (Figure 2). Furthermore, a reversal of tubular epithelial cells degeneration seen in the nephron of infected-untreated chickens was observed with treatment groups 3 and 4 at 200 and 400 mg/kg respectively (Figure 3).

Discussion

This study reported the in-vitro and *in-vivo* antibacterial activity of *Lagenaria breviflora* whole fruit against *Salmonella species. T*he minimum inhibitory concentration (100 µg/ml) in culture media, as well as the minimum bacteriocidal concentration (200 µg/ml) in broth culture, were established. These findings correlated with an earlier report (Tomori et al., 2007) which documented the antibacterial effect of *L. breviflora* in both gram-negative and positive organisms, including *S. typhi, S. paratyphi* and *S. dysenteriae*. These organisms cause infections clinically presented as gastroenteritis and are implicated in wound sepsis (Banjo et al., 2013). *In-vivo* study in experimentally infected chickens showed the antibacterial potential of *L. breviflora* methanol extracts against *Salmonella sp*. The bacteria colony counts showed an appreciable ($p<0.05$) decline in gut bacteria growth of all chickens that received treatment in the 10-day course of infection. This supports a previous report which stated that the plant had demonstrated antibacterial potentials in *in-vitro* studies (Tomori et al., 2007). The mechanism of bacteriocidal action is, however, unknown.

Furthermore, infected-untreated chickens in this study had elevated PCV, beyond the documented PCV range of 28.67 - 30.33% in healthy broiler chickens (Arogbodo et al., 2020). This indicated dehydration in these untreated chickens, which is characteristic of diarrhea seen in salmonellosis (Nabil et al., 2018). Elevated red cell indices of infected-untreated chickens also affirmed this clinical finding but were reversed in the infected chickens treated with the extract or ciprofloxacin. This corroborated a previous study on the relaxant effect of *L. breviflora* fruit extract on the rabbit ileal smooth muscle, indicative of its anti-diarrhoeic effect (Oridupa and Saba, 2013).

The leucogram showed leukocytosis with left-shift neutrophilia in infected chickens, typical of a bacterial infection (Stacy et al., 2022). Histology of the liver and kidney of infected-untreated chickens revealed acute inflammation which can be correlated with ongoing systemic bacterial infection with consequential influx of inflammatory cells (Amor et al., 2014). However, *L. breviflora*-treated chickens had normal leucogram and cellular architecture of the organ. Platelet count in infected chicks treated with extract was similar to healthy chicks in this study, while there was thrombocytosis in infected-untreated chicks. This clinical presentation (thrombocytosis) coupled with dehydration predisposes to disseminated intravascular coagulopathies amongst other bleeding disorders associated with Salmonellosis (Iba et al., 2022).

Table 5: Proteins and liver enzymes of chickens (healthy, experimentally infected with *Salmonella sp* either untreated or treated with methanol extract of *Lagenaria breviflora* whole fruit or ciprofloxacin)

	TRT1	TRT2	TRT3	TRT4	TRT5	TRT6
TRT	CONTROL	Infected-Untreated	200 mg/kg LB + Salmonella	400 mg/ kg LB + Salmonella	800 mg/ kg LB + Salmonella	20 mg/ kg Cipro + Salmonella
TP (g/dL)	6.87 ± 0.12	6.63 ± 0.12	7.00 ± 0.12	7.13 ± 0.09	6.53 ± 0.09	6.83 ± 0.15
ALB (g/dL)	3.77 ± 0.12	3.67 ± 0.09	3.93 ± 0.88	4.03 ± 0.12	3.50 ± 0.06	3.80 ± 0.15
GLOB (g/dL)	3.10 ± 1.81	2.97 ± 0.07	3.07 ± 0.03	3.10 ± 0.06	3.03 ± 0.03	3.03 ± 0.03
A/G RATIO	1.22 ± 0.04	1.24 ± 0.04	1.28 ± 0.02	1.30 ± 0.06	1.15 ± 0.01	1.25 ± 0.0
ALP (IU/L)	53.00 ± 3.21	61.33 ± 0.88	62.00 ± 1.53	52.00 ± 0.58	49.00 ± 1.53	43.67 ± 0.88[a]
AST (IU/L)	14.00 ± 1.15	15.67 ± 1.76	13.67 ± 0.88	15.00 ± 1.00	16.00 ± 1.15	12.67 ± 0.67
ALT (IU/L)	11.00 ± 0.58	11.67 ± 0.88	11.00 ± 0.58	12.67 ± 0.67	12.67 ± 1.45	9.67 ± 0.88
BIL(mg/dL)	0.40 ± 0.06	0.70 ± 0.12	0.77 ± 0.03	0.40 ± 0.12	0.47 ± 0.06	0.63 ± 0.09
BIL (mg/dL)	0.23 ± 0.03	0.27 ± 0.07	0.37 ± 0.07	0.23 ± 0.03	0.23 ± 0.03	0.30 ± 0.06
BIL (mg/dL)	0.17 ± 0.03	0.43 ± 0.07	0.40 ± 0.06	0.17 ± 0.12	0.23 ± 0.07	0.33 ± 0.09

Mean±standard deviation; Values with superscript ([a]) are statistically significant ($p<0.05$) compared to control. Values with superscript ([b]) are statistically significant ($p<0.05$) compared to infected-untreated.

Table 6: Lipid and renal parameters of chickens (healthy, experimentally infected with Salmonella sp either untreated or treated with methanol extract of Lagenaria breviflora whole fruit or ciprofloxacin)

	TRT1	TRT2	TRT3	TRT4	TRT5	TRT6
TRT	CONTROL	Infected-Untreated	200 mg/kg LB + Salmonella	400 mg/ kg LB + Salmonella	800 mg/ kg LB + Salmonella	20 mg/ kg Cipro + Salmonella
TC (mg/dL)	95.00 ± 7.63	49.33 ± 1.76	68.00 ± 3.46[ab]	65.00 ± 1.73[b]	62.33 ± 1.45[b]	75.67 ± 3.48[ab]
TG (mg/dL)	27.67 ± 1.86	18.33 ± 1.76	25.00 ± 2.65	25.00 ± 1.15	26.67 ± 2.33	27.33 ± 3.84
HDL (mg/dL)	17.00 ± 2.08	10.33 ± 0.33	14.33 ± 1.45	15.00 ± 0.58	16.00 ± 2.08	15.00 ± 2.52
LDL (mg/dL)	54.00 ± 5.29	27.67 ± 2.60	39.67 ± 3.93	39.33 ± 4.41[b]	37.00 ± 2.65	37.67 ± 4.41
AIP	0.22 ± 0.03	0.25 ± 0.04	0.24 ± 0.04	0.23 ± 0.05	0.23 ± 0.04	0.26 ± 0.06
UREA (mg/dL)	27.67 ± 1.45	37.67 ± 1.45	30.00 ± 1.15	32.00 ± 1.53	29.67 ± 1.45	27.67 ± 1.45[b]
CRT (mg/dL)	0.63 ± 0.03	1.07 ± 0.03	0.70 ± 0.06	0.70 ± 0.06	0.71 ± 0.03	0.60 ± 0.06

Mean±standard deviation; Values with superscript ([a]) are statistically significant ($p<0.05$) compared to control. Values with superscript ([b]) are statistically significant ($p<0.05$) compared to infected-untreated.

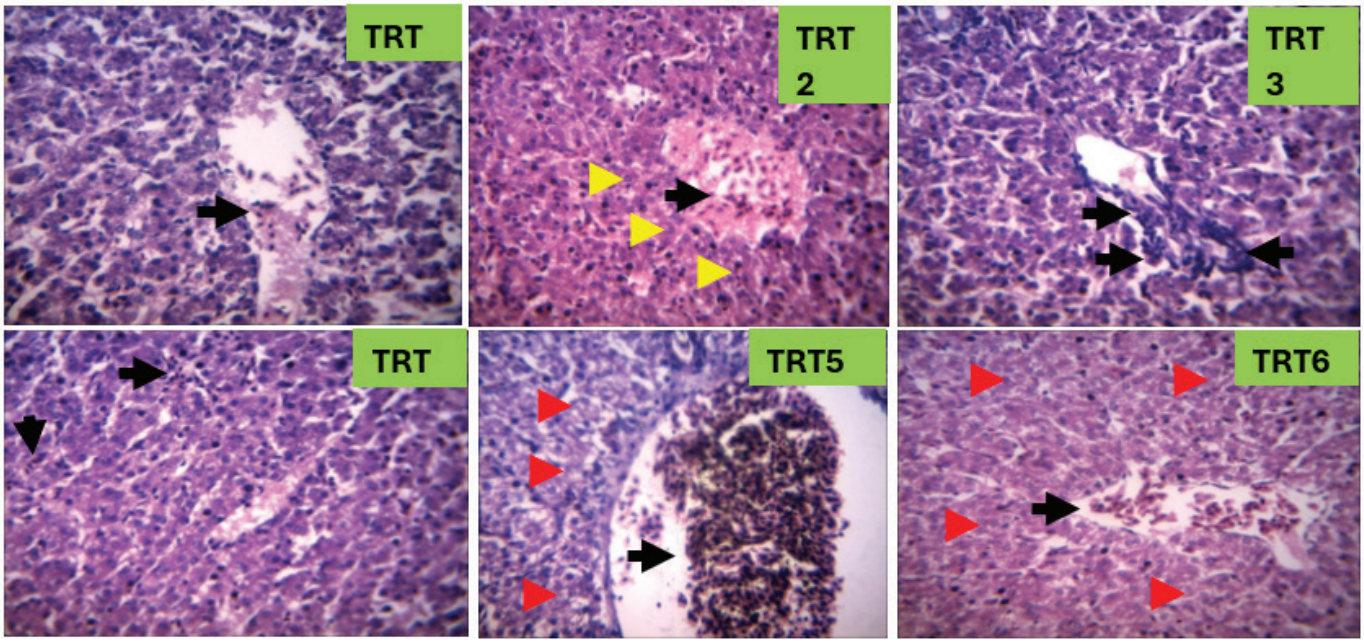

Figure 2: Liver of chickens (healthy, experimentally infected with Salmonella sp either untreated or treated with methanol extract of Lagenaria breviflora whole fruit or ciprofloxacin) (H&E, X100)

TRT1 (Control)- Very mild portal congestion (red arrow).

TRT2 (Infected-Untreated)- Moderate portal congestion (black arrow), with mild periportal hydropic degeneration of hepatocytes (yellow arrowheads).

TRT3 (200 mg/kg LB + Salmonella)- Very mild sinusoidal congestion (black arrows).

TRT4 (400 mg/kg LB + Salmonella)- Very mild sinusoidal congestion (black arrows).

TRT5 (800 mg/kg LB + Salmonella) - Moderate diffuse vacuolar degeneration of hepatocytes (red arrowheads), with a severe portal congestion (black arrow).

TRT6 (20 mg/kg Cipro + Salmonella)- Very mild diffuse hydropic degeneration of hepatocytes (red arrowheads) and portal congestion (black arrow).

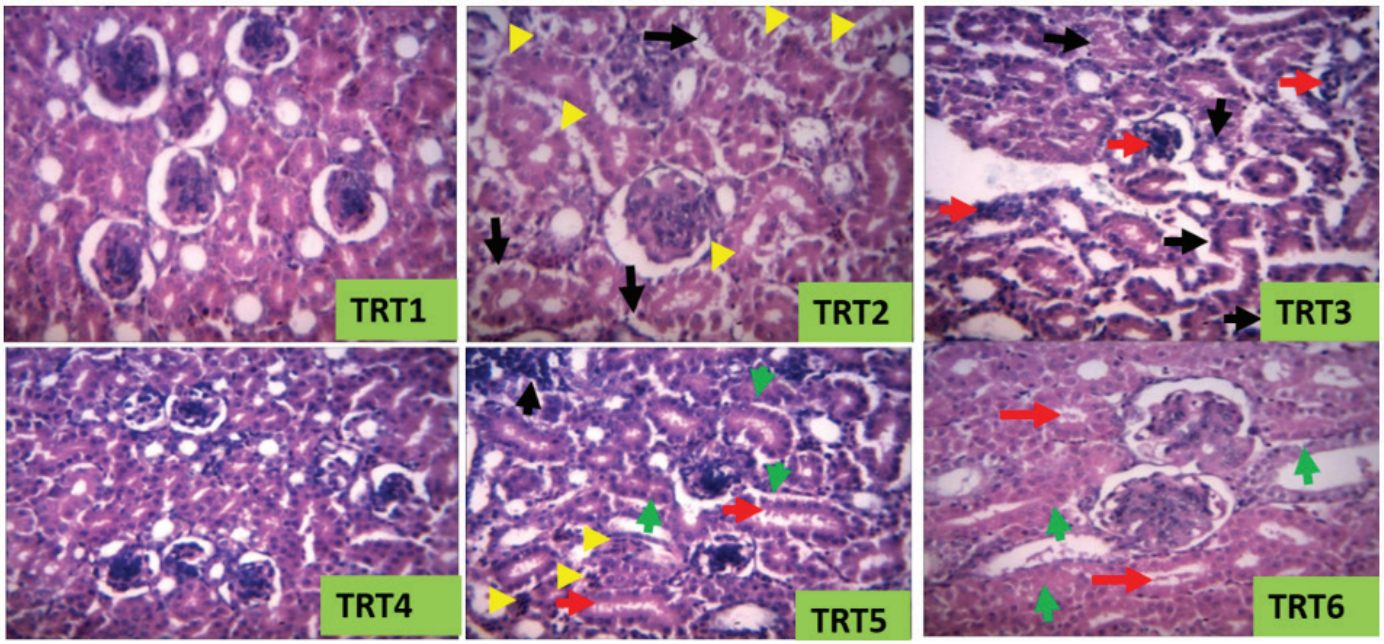

Figure 3: Kidney of chickens (healthy, experimentally infected with Salmonella sp either untreated or treated with methanol extract of Lagenaria breviflora whole fruit or ciprofloxacin) (H&E, X100)

TRT1(Control)- No visible lesions seen

TRT2 (Infected-Untreated)- There is detachment of tubular epithelium from the basement membrane (black arrow) and degeneration of some of the tubular epithelial cells (yellow arrowhead)

TRT3 (200 mg/kg LB + Salmonella)- Mild to moderate glomerular (red arrow) and tubular degeneration (black arrow).

TRT4 (400 mg/kg LB + Salmonella) - No visible lesions seen.

TRT5 (800 mg/kg LB + Salmonella)- Mild interstitial cellular infiltration (black arrows). Brush borders clumping with intraluminar debris in the proximal convoluted tubules, mild congestion (yellow arrow) & nuclear degenerative changes (green arrow).

TRT6 (20 mg/kg Cipro + Salmonella)- No visible lesions seen. Tissue debris/casts in the urinary tubule (red arrow) with some level of nuclear degeneration (green arrow)

This disease is also accompanied by hepatic and renal derangement clinically exhibited as hepatomegaly, hepatic parenchyma infiltration, hepatic necrosis/ degeneration, and hepatitis due to infiltration inflammatory cells, as well as nephritis and tubular necrosis of the kidneys (Muna et al., 2016; Shallal, 2018). Diagnosis of hepatic and renal injury is usually made by measurements of liver enzymes, urea, and creatinine clearance (Pandya et al., 2016). Although liver enzymes, total protein, and its constituent fractions were unchanged in this study, liver histopathology in infected-untreated chickens revealed inflammatory cell infiltration, suggestive of bacteria localization (Saleem et al., 2022) in this organ but was reversed in treated chickens.

On the other hand, renal derangement observed in infected-untreated chickens was also inhibited by the extract in treated chickens. The observable increase of serum urea, creatinine, total and direct bilirubin was reversed in *L. breviflora* -treated birds in this study, accounting for the reversal of renal damage caused by *Salmonella species*. Severe liver and kidney damage has been reportedly associated with organ/systemic failure in birds infected with salmonellosis (Nazir et al., 2012). According to this current study, renal impairment expressed as mild to moderate degeneration of nephrocytes and glomeruli at histology, was ameliorated at 200mg/kg and 400mg/kg of the *L. breviflora* extract.

In conclusion, this study established the *in-vitro* and *in-vivo* antibacterial effect of *L. breviflora* whole fruit methanol extract against salmonellosis in chickens. However, the mechanism of bacteriocidal action is yet to be determined. Reversal of clinical signs of Salmonellosis presented as diarrhea, hypovolaemia, and dehydration confirmed the antidiarrhoeic properties of this extract. In addition, *L. breviflora* extract ameliorated hepatic and renal damage associated with salmonellosis, as seen by restoration of biochemical parameters and organ architecture in extract-treated chickens. Therefore, *L. breviflora* whole fruit is a potential antibacterial drug candidate against salmonellosis, a common bacterial infection accounting for significant economic losses in the poultry industry. This will advance the campaign for a reduction in synthetic antibiotic use, responsible for antimicrobial resistance and drug residues in food animals.

Acknowledgment

The authors acknowledge the effort of Mr. Yemi Okunlade of the Department of Veterinary Public Health and Preventive Medicine Laboratory, University of Ibadan for the support given throughout this research and the management of Bora Farms, I.A.R.&T, Obafemi Awolowo University, Ibadan for allowing us to use their poultry facilities in the course of the research.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

Abd El-Hack ME, El-Saadony MT, Elbestawy AR, Nahed A, Saad AM, Salem HM., El-Tahan AM, Khafaga AF, Taha AE, AbuQamar SF, El-Tarabily KA (2022). Necrotic enteritis in broiler chickens: disease characteristics and prevention using organic antibiotic alternatives–a comprehensive review. Poultry Science, 101(2): 101590.

Aćimović MG, Tešević VV, Katarina T, Smiljanić TS, Cvetković MT, Stanković JM, Kiprovski BM, Sikora VS, (2020). Hydrolates – By-Products of Essential Oil Distillation: Chemical Composition, Biological Activity and Potential Uses. Advanced Technologies, 9(2): 54-70.

Adeyemi MA, Ekunseitan DA, Abiola SS, Dipeolu MA, Egbeyale LT, Sogunle OM (2017). Phytochemical analysis and GC-MS determination of Lagenaria breviflora R. fruit. International Journal of Pharmacognosy and

Phytochemical Research, 9(7): 1045-1050.

Amor S, Peferoen LA, Vogel DY, Breur M, van der Valk P, Baker D, van Noort JM (2014). Inflammation in neurodegenerative diseases–an update. Immunology, 142(2): 151-166.

Audisio MC, Terzolo HR (2002). Virulence analysis of a Salmonella gallinarum strain by oral inoculation of 20-day-old chickens. Avian Diseases, 46(1): 186-191.

Arogbodo JO, Osho IB, Faluyi OB, Awoniyi TAM (2020). Haematological indices of Salmonella gallinarum (Gr. D1-1, 9, 12) infected broiler chickens treated with ethanolic leaf extract of Chrysophyllum albidum (G. Don). Nigerian Journal Animal Production, 47(1): 65-80.

Banjo TA, Kasim LS, Iwalokun BA, Mutiu WB, Olooto WE, Mba NG, James ES, Shorunmu TO (2013). Effects of different extraction methods on in-vitro antimicrobial properties of Lagenaria breviflora whole Fruits. New York Science Journal, 6(10): 60-65.

Carvalho IT, Santos L (2016). Antibiotics in the aquatic environments: a review of the European scenario. Environment International, 94: 736-757.

CLSI (2018). Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals. 5th ed. Wayne, PA: Clinical and Laboratory Standards Institute.

Dhillon NP, Sanguansil S, Singh SP, Masud MAT, Kumar P, Bharathi LK, Halit Y, Rukui H, Doan XC, McCreight JD (2016). Gourds: bitter, bottle, wax, snake, sponge and ridge. Genetics and genomics cucurbitaceae, 1-18.

Durmic Z, Blache D (2012). Bioactive plants and plant products: Effects on animal function, health and welfare. Animal Feed Science and Technology, 176: 150-162.

Forgetta V, Rempel H, Malouin F, Vaillancourt Jr R, Topp E, Dewar K (2012). Pathogenic and multidrug-resistant Escherichia fergusonii from broiler chicken. Poultry Science, 91: 512-525.

Gonzalez Ronquillo M, Angeles Hernandez JC (2017). Antibiotic and synthetic growth promoters in animal diets: review of impact and analytical methods. Food Control, 72: 255-267.

Iba T, Connors JM, Levi M, Jerrold LH (2022). Heatstroke-induced coagulopathy: Biomarkers, mechanistic insights, and patient management. ClinicalMedicine, 44: 1-9.

Kostadinović L, Puvača N, Popović S, Lević J (2015). Botanical supplements as anti-coccidial alternatives in poultry nutrition. World's Poultry Science Journal, 71(1): 27-36.

Kowalska-Krochmal B, Dudek-Wicher R (2021). The minimum inhibitory concentration of antibiotics: Methods, interpretation, clinical relevance. Pathogens, 10(2): 165.

Lala V, Zubair M, Minter DA (2023). Liver Function Tests. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; Jan-. [Updated 2023 Jul 30]. Available online at: https://www.ncbi.nlm.nih.gov/books/NBK482489/.

Lorke D (1983). A new approach to practical acute toxicity testing. Archives of Toxicology, 54: 275–87.

Muna EA, Salih MH, Zakia AM, Halima MO, Abeer AM, Ameera MM, Huda OA, Idris SB (2016). Pathology of Broiler Chicks Naturally Infected with Salmonella enteritidis (S. enteritidis) & Salmonella typhimurium (S. typhimurium) During an Outbreak in Sudan. Journal of Scientific Research and Reports, 10(1): 1-8.

Nazir S, Kamil SA, Darzi MM, Mir M, Saleem N, Reda A (2012). Pathology of Spontaneously Occurring Salmonellosis in Commercial Broiler Chickens of Kashmir Valley. Journal of World's Poultry Research, 2: 63-69.

Nabil NM, Tawakol MM, Hassan HM (2018). Assessing the impact of bacteriophages in the treatment of Salmonella in broiler chickens.

Infectious Ecology and Epidemiology, 8(1): 1539056.

Oridupa OA, Saba AB (2013). Relaxant effect of Lagenaria breviflora Roberty fruit pulp and seeds on isolated rabbit ileum. Sokoto Journal of Veterinary Science, 11(2): 21-27.

Oridupa O, Ojojugbo F, Ovwighose N (2018). Haematological and Biochemical Changes Associated with Treatment of Experimentally-Induced Hypertensive Wistar Rats with Lagenaria breviflora Roberty Fruit or Xanthosoma sagittifolium Exell Corm. Annual Research and Review in Biology, 26(5): 1-8.

Pandya D, Nagrajappa AK, Ravi KS (2016). Assessment and correlation of urea and creatinine levels in saliva and serum of patients with chronic kidney disease, diabetes and hypertension–a research study. Journal of Clinical Diagnostic Research, 10(10): ZC58-ZC62.

Puvača N, Stanaćev V, Glamočić D, Lević J, Perić L, Stanaćev V, Milić D (2013). Beneficial effects of phytoadditives in broiler nutrition. World's Poultry Science Journal, 69: 27-34.

Roger T, Pierre-Marie M, Igor VK (2015). Phytochemical screening and antibacterial activity of medicinal plants used to treat typhoid fever in Bamboutos division, West Cameroon. Journal of Applied Pharmaceutical Science, 5(6): 034-049.

Ruiz J, Nunez ML, Lorente I, Perez J, Simarro E, Gomez J (1996). Performance of six culture media for isolation of Salmonella species from stool samples. European Journal of Clinical Microbiology and Infectious Diseases, 15: 922-926.

Saba AB, Oridupa OA, Oyagbemi AA, Alao EO (2010). Serum biochemical changes accompanying prolonged administration of ethanolic extract of whole fruit of Lagenaria breviflora (Benth) Roberty in Wistar rats. African Journal of Biotechnology, 9(42): 7128-7133.

Saeed M, Khan MS, Amir K, Bi JB, Asif M, Madni A, Kamboh AA, Manzoor Z, Younas U, Chao S (2022). Lagenaria siceraria fruit: A review of its phytochemistry, pharmacology, and promising traditional uses. Frontiers in Nutrition, 9: 927361.

Saleem G, Farooq U, Naseer R, Aslam HB, Mustafa G, Omar MO, Liaqat I (2022). Pathobiological and Immunohistochemical Findings in Broiler Chickens Naturally Infected with Salmonella Enterica Serotype Gallinarum Biotype Gallinarum. Pakistan Veterinary Journal, 42(1): 88-94.

Shallal ZS (2018). Pathology of internal organs after infection of mice experimentally with LD50 dose of Salmonella mbandaka through the oral route. Journal of Entomology and Zoology Studies, 6(1): 469-472.

Stacy NI, Hollinger C, Arnold JE, Cray C, Pendl H, Nelson PJ, Harvey JW (2022). Left shift and toxic change in heterophils and neutrophils of non-mammalian vertebrates: A comparative review, image atlas, and practical considerations. Veterinary Clinical Pathology, 51(1): 18-44.

Van Hoogevest P, Wendel A (2014). The use of natural and synthetic phospholipids as pharmaceutical excipients. European Journal of Lipid Science and Technology, 116(9): 1088-1107.