



RESEARCH ARTICLE

Study on the inhibitory effects of guava leaves extract in broiler chickens experimentally challenged with *Salmonella gallinarum*

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ABSTRACT

The current study aimed to explore the phytochemical composition of guava (*Psidium guajava*) leaves extract and to assess its antimicrobial activity. This was done by *in vitro* assays, such as agar well diffusion assay and minimum inhibitory concentration (MIC) and *in vivo* antimicrobial testing in broiler chickens experimentally infected with *Salmonella gallinarum* (SG). Phytochemical screening showed the presence of flavonoids, tannins, terpenoids, phenols, and saponins. The zone of inhibition (ZOI) of guava leaves extract against SG according to the agar well diffusion assay was concentration-dependent. MIC was found to be 6.25 mg/mL. In the case of the *in vivo* study, 150 day-old broiler chicks were obtained and separated into five groups (A, B, C, D, and E). Group A was the control group that was not infected. Group B (preventive) was treated with the extract on days 4, 5 and 6 and then inoculated with SG (3×10^8 CFU/mL) on day 7. Group C: SG was infected but untreated. On day 7, group D was inoculated with SG and once clinical symptoms were experienced, the extract of guava leaves was used on days 11, 12 and 13. On day 7, Group E was inoculated and on days 11, 12 and 13 the standard antibiotic ciprofloxacin was administered. Hematological and serum biochemical evaluations on days 7, 14, and 21 demonstrated that guava leaves extract exerted a potent antimicrobial effect against *Salmonella gallinarum*, with efficacy comparable to the standard antibiotic ciprofloxacin in the *in vivo* model. All treated groups had their severity of gross and histopathological changes reduced by guava leaves extract. It is interesting to note that the conventional antibiotic ciprofloxacin was less effective in *in vitro* and *in vivo* analyses. The conclusion was that guava leaves extract is a strong antimicrobial agent against *Salmonella gallinarum* in broiler chickens.

Keywords: Guava leaves; Broiler Chickens; *Salmonella gallinarum*.

INTRODUCTION

In Pakistan, poultry farming started in the 1960s and has shown a high rate of growth of 20-30 percent per year since then, mainly due to demand in the domestic market (Hussain *et al.*, 2015). In Pakistan, poultry production is an important branch of the food processing industry. The poultry sector has registered impressive growth, with meat production increasing by 8.1% over the past decade. Pakistan has emerged as the 11th largest poultry producer globally. The poultry industry contributes 43.3% of the total meat production in the country. In 2024-25, the livestock sector as a whole contributed 63.6% to agriculture's value addition and 14.97% to the national GDP (Government of Pakistan, 2025).

Salmonella is a foodborne zoonotic pathogen of the family Enterobacteriaceae, which includes over 2,600 serotypes. It is a

Gram-negative, facultative anaerobic, rod-shaped bacterium that is commonly present in the gastrointestinal tract (GIT) of birds and animals. The majority of *Salmonella* are motile (Kumar *et al.*, 2025). Non-motile serotypes like *Salmonella gallinarum* have been identified to cause salmonellosis in poultry and cause huge losses in the poultry industry (El-Shall *et al.*, 2026). The disease is important because of the vertical transmission of the causal agent between parent stock and offspring (Li *et al.*, 2021). There are two main biovars of *Salmonella enterica* subsp. *enterica* Gallinarum biovar Gallinarum, the cause of fowl typhoid, and Gallinarum biovar Pullorum, the cause of pullorum disease. Although mostly limited to the GIT, invasive forms are sometimes involved in clinical salmonellosis (Wales & Lawes, 2023; Farhat *et al.*, 2023).

Fowl typhoid is a septicemic infection of chickens, turkeys, ducks and other birds. *Salmonella gallinarum*, the causative agent, was

initially identified in 1888 and has a worldwide distribution, but has been eliminated in commercial flocks in the USA, Canada, Australia, Japan and some of Western Europe. The morbidity and mortality are variable and depend on the host age, nutritional status, flock management and concurrent infections with a mortality rate of 0 to 100 percent (Shivaprasad, 2000).

Clinical manifestations of fowl typhoid include greenish-yellow diarrhea, and enlargement and congestion of the kidney, liver, and spleen (Shafi *et al.*, 2025). The liver often exhibits 2-4 mm necrotic foci. Histologically, infiltration of hepatocytes with plasma cells and lymphocytes is observed (Tiwari *et al.*, 2021). In developed countries, fowl typhoid has been controlled through serological testing and the elimination of reactor birds (Shivaprasad, 2000). Bacterial colonization and multiplication within tissues can lead to acute mortality shortly post-infection, marked by profound hepatomegaly (Tiwari *et al.*, 2021).

Although antibiotics are a traditional approach to managing salmonellosis, overuse has resulted in antimicrobial resistance (Lamichhane *et al.*, 2024). Today, there are no effective immunoprophylactic measures that are broadly used, and tight biosecurity remains one of the main forms of prevention (Raut *et al.*, 2023). The constant application of antibiotics leads to the development of resistance in poultry and has residual effects in consumers. Consequently, the world is striving to come up with alternative measures that would offer immunity against *Salmonella* and other pathogens. The environment does not readily lose salmonella and this makes it hard to control the disease (Sana *et al.*, 2025). Control was previously obtained using antimicrobial growth promoters (AGPs), which can be used routinely, but they can cause cross-resistance among microbial populations (Abd El-Hack *et al.*, 2022). Vaccines, bacteriophages, immunomodulatory agents, and plant-derived phytobiotics are also promising substitutes to AGPs (Abd El-Hack *et al.*, 2022). Polyphenols and tannins, essential oils, lectins and polypeptides, and alkaloids are the major antimicrobial phytochemicals in these plant extracts (Kuralkar & Kuralkar, 2021). The use of phytobiotics as feed additives is considered safe and effective due to their antimicrobial, anti-parasitic, anti-inflammatory, and antioxidant effects (Obianwuna *et al.*, 2024; Urban *et al.*, 2025).

The presence of bioactive compounds including tannins, saponins, flavonoids, and triterpenoids make guava leaves traditionally used as medicine in the treatment of diarrhea and thrush (Khanna *et al.*, 2025). Tannins are high molecular weight polyphenolic compounds that form complexes with proteins and add to the antibacterial effects of guava leaves. Research has shown that tannin concentration increases the antibacterial activity. Early research has established the antibacterial properties of guava leaves extract against *Escherichia coli*, with 40% concentration being very effective (Huynh *et al.*, 2025; Mailoa *et al.*, 2014). Thus, the current experiment aimed to evaluate the antibacterial properties of guava leaves extract on *Salmonella gallinarum*.

MATERIALS AND METHODS

Guava leaves collection

The leaves of guava were picked in the city of Sheikhupura, Pakistan, and placed in plastic zipper bags (ice-packed) and shipped to the Bacteriology Laboratory, Department of Pathology, University of Veterinary and Animal Sciences (UVAS), Lahore.

In vitro study

Extraction of Guava Leaves Extract

Guava leaves were dried in a hot air oven and blended into a fine powder. Fifty grams of powdered guava leaves were added to a flask containing 250 mL of methanol solution and incubated in a shaker incubator at 30°C for 3 days. The extract was filtered using Whatman No. 1 filter paper. Methanol was evaporated from the filtrate using

a rotary evaporator at 60°C. The resulting evaporated methanolic extract was cooled in a refrigerator and stored for further processing.

Phytochemical screening

Qualitative chemical tests were performed to identify the presence of bioactive components in the guava leaves extract, following the methodology described by Shaikh and Patil (2020).

- Saponins: 4 mL of extract was combined with 5–6 drops of olive oil in a test tube. The formation of a stable foam emulsion indicated the presence of saponins.
- Phenols and Tannins: 4 mL of extract was mixed with 3 mL of 2% ferric chloride (FeCl_3) in a test tube. The appearance of a blue-black color indicated the presence of phenols and tannins.
- Terpenoids: 2 mL of chloroform and 2 mL of concentrated sulfuric acid were added to 2 mL of guava leaves extract. The formation of a reddish-brown color indicated the presence of terpenoids.
- Flavonoids: Magnesium fragments were mixed with the extract, and concentrated hydrochloric acid was added dropwise. The appearance of a red, orange, or pink color indicated the presence of flavonoids.

High Performance Liquid Chromatography (HPLC)

HPLC analysis was performed at the WTO Laboratory, UVAS, Lahore. Gallic acid, sinapic acid, and ferulic acid were used as reference standards.

Bacterial Culture

Preserved isolates of *Salmonella gallinarum* were transferred to Buffered Peptone Water and incubated overnight at 37°C for 24 hours. One milliliter of the incubated pre-enrichment broth was transferred into Selenite broth and incubated for 24 hours at 37°C. A loopful from the broth was streaked onto *Salmonella*-*Shigella* (S.S) agar plates and incubated for 24 hours at 37°C.

Antibacterial Sensitivity Test (Agar Well Diffusion)

The antibacterial activity of guava leaves extract was evaluated using the agar well diffusion method on Nutrient Agar plates. Four wells (approx. 5 mm diameter) were punched into each plate using a sterile borer. A 0.5 McFarland standard suspension of *Salmonella gallinarum* was prepared and swabbed onto the plates using a sterile cotton swab. Plates were allowed to dry for 3 minutes. Each well received 0.5 mL of extract at different concentrations (100 mg/mL, 200 mg/mL, 300 mg/mL, and 400 mg/mL). A ciprofloxacin disk served as the positive control. Plates were incubated at 37°C for 24 hours, after which the zones of inhibition were measured.

Minimum Inhibitory Concentration (MIC)

The MIC of guava leaves extract was ascertained through the broth microdilution technique on a 96-well microtiter plate. Nutrient Broth (50 mL) was dispensed into wells 1 through 12. A standardized *Salmonella gallinarum* suspension (0.5 McFarland, 10^8 CFU/mL) was added to wells 1 through 11. The extract of the guava leaves (100 mg/mL) was diluted two-fold in series, beginning with well 1 up to well 10. The plate was incubated at 37°C, 24 hours. An ELISA was used to measure the optical density (OD) at 595 nm before and after incubation. Wells 11 and 12 were used as negative and positive controls, respectively.

In vivo study

Experimental Design

A total of One hundred and fifty (150) day-old broiler chicks (DOCs) were purchased at a well-established hatchery. The chicks were kept in hygienic conditions, fed a balanced ration as well as given clean water *ad libitum*. No antibacterial agents were given before or during the experiment, unless prescribed by the study design.

Salmonella spp. were randomly screened in birds through cloacal swabs in Selenite broth and streaked on S.S agar to verify that there is no infection before the trial. Birds were randomly divided into five groups (A, B, C, D and E) with three replicas of 10 birds in each group. The experimental timeline was designed based on preliminary pilot studies and established protocols for *Salmonella gallinarum* challenge models in poultry (Kumari et al., 2013; Sannat et al., 2017). The day 7 sampling point was scheduled prior to challenge to establish baseline physiological, hematological, and serum biochemical values for each experimental group, ensuring that any observed differences at later time points could be attributed to the infection and/or treatment interventions rather than pre-existing variations between groups. The day 14 sampling (7 days post-challenge) was selected to capture the acute phase of infection, when clinical signs and pathological changes are most pronounced based on our pilot studies and previous reports (Chiroma et al., 2018; Tiwari et al., 2021). The day 21 sampling (14 days post-challenge and 7 days after treatment completion) was specifically chosen to evaluate the sustainability and durability of the therapeutic effect, assessing whether the beneficial effects of guava leaves extract persist after treatment withdrawal—a clinically relevant parameter for practical field applications where continuous supplementation may not be economically or logistically feasible. Treatment initiation on days 11-13 post-challenge was based on pilot studies in which clinical signs of fowl typhoid (depression, huddling, ruffled feathers, anorexia, and creamy whitish diarrhea) consistently manifested between days 9 and 11 following challenge at the given dose. This timeline mimics a realistic field scenario where treatment would be initiated upon recognition of clinical illness rather than being administered prophylactically, thereby providing a more clinically applicable assessment of therapeutic efficacy.

Ethical Approval

All experimental procedures were approved by the Institutional Guidelines for the Care and Use of Laboratory Animals of the University of Veterinary and Animal Sciences, Lahore (Approval No. DR/752, Dated: 17-7-2024).

Challenge and Treatment Dose

The infection of *S. gallinarum* was done through crop gavage with a dose of 0.2 mL of sterile normal saline containing about 3×10^8 CFU/mL. Guava leaves extract was added to the drinking water at a concentration of 6.25 mg/mL. This concentration was selected based on the *in vitro* minimum inhibitory concentration (MIC) value of 6.25 mg/mL determined in this study, with a 1× MIC margin applied. Based on average daily water consumption (150-200 mL/bird/day) and average body weight during the treatment period (approximately 450-550 g), the estimated daily intake was approximately 10-12.5 mg/kg body weight. Ciprofloxacin was administered orally via crop gavage at a dosage of 10 mg/kg body weight, once daily, on days 11, 12, and 13 post-infection. The dosage was based on the British Pharmacopoeia veterinary guidelines and was consistent with previous studies on poultry salmonellosis (Sannat et al., 2017).

Experimental Groups

The birds were randomly assigned to five experimental groups each having 30 birds with three replicas of 10 birds each. Group A was the negative control and it was not challenged or treated in the period between days 1 and 21. Group B was the preventive group; these birds were given guava leaves extract in their drinking water on day 4, 5 and 6, and then subjected to experimental challenge with *S. gallinarum* on day 7. Group C was the positive control (infected, untreated) and was challenged with *S. gallinarum* on day 7 and no further therapeutic intervention was administered. Treatment group was group D; birds were tested against *S. gallinarum* on day 7 and on the day when clinical signs appeared guava leaves extract was added on days 11, 12, and 13 after infection. Group E was used as

an antibiotic control, the birds were challenged with *S. gallinarum* on day 7 and ciprofloxacin was administered to the birds on day 11, 12, and 13.

Feed Conversion Ratio (FCR)

Feed Conversion Ratio was calculated on the final day of the experiment and compared against standard broiler performance metrics.

Serum Biochemistry

Blood samples (approximately 3 mL) were collected from the brachial vein of four birds per group on days 7, 14, and 21 (corresponding to baseline, acute phase, and post-treatment follow-up, respectively) into serum separator tubes (SSTs). The commercial colorimetric kits were used to measure serum levels of Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), and Aspartate Aminotransferase (AST).

Hematological Estimation

On days 7, 14, and 21, six birds per group were randomly selected, and 3 mL of blood was collected from the wing vein into EDTA vacutainers. Red Blood Cell (RBC) count, White Blood Cell (WBC) count, and Hemoglobin (Hb) concentration were measured. Hemoglobin was estimated using the Drabkin method (Drabkin & Austin, 1935).

Histopathological Examination

Necropsy was performed to evaluate the effect of treatment on challenged birds. Tissue samples (liver, spleen, and intestine) were collected and fixed in 10% buffered formalin. Tissues were processed using standard histological techniques and stained for microscopic examination (Shaukat et al., 2025).

Statistical Analysis

Data were analyzed using SPSS version 20. One-way analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test was used for multiple group comparisons. Normality and homogeneity of variances were assessed using Shapiro-Wilk and Levene's tests, respectively. Non-normally distributed data were analyzed using the Kruskal-Wallis test. Quantitative variables are presented as mean $\pm$ SD, and differences were considered significant at $P < 0.05$.

RESULTS

In vitro study

Extraction and Phytochemical Analysis

Methanolic extraction of 50 g of guava leaves powder yielded approximately 6 g of extract. Phytochemical analysis confirmed the presence of phenols, tannins, saponins, terpenoids, and flavonoids (Table 1). High-performance liquid chromatography (HPLC) analysis was performed to identify phenolic compounds in the extract. The chromatographic profile confirmed that the guava leaves extract was positive for gallic acid (Figure 1A and Figure 1B).

Antibacterial Sensitivity and MIC

The extract demonstrated concentration-dependent antibacterial activity, with zones of inhibition ranging from 16 mm to 27 mm

Table 1. Phytochemical screening of methanolic guava leaves extract

Test	Result
Saponins	+
Phenols and Tannins	+
Terpenoids	+
Flavonoids	+

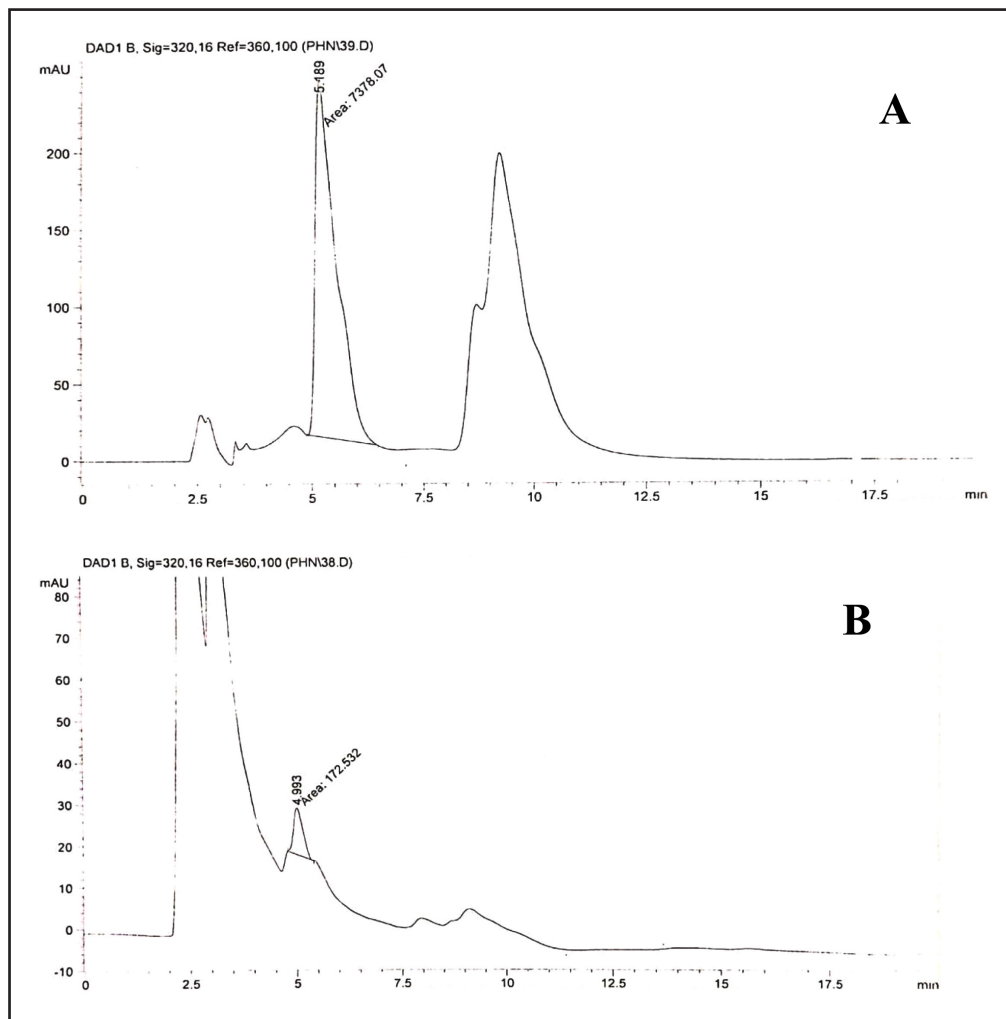


Figure 1. High-performance liquid chromatography (HPLC) profiles of methanolic guava leaves extract. (A) Chromatogram showing detection of gallic acid and other phenolic constituents. (B) Expanded chromatogram confirming the presence of gallic acid as a major phenolic compound. Standards used: gallic acid, sinapic acid, and ferulic acid.

(Table 2 and Figure 2). The minimum inhibitory concentration was determined to be 6.25 mg/mL, as bacterial growth was observed in wells with lower extract concentrations (Table 3).

In vivo study

Serum Biochemistry

At day 7 (baseline, prior to challenge), there were no significant differences ($P > 0.05$) in serum biochemical or hematological parameters between the control group and the guava extract-supplemented preventive group, confirming the absence of adverse hepatotoxic or hematological effects of the extract and validating the comparability of groups prior to infection (Table 4).

At day 14 (7 days post-challenge, acute phase), birds in Group C (infected, untreated) exhibited markedly elevated liver enzymes and significant declines in hemoglobin and RBC counts compared to all other groups ($P < 0.05$), confirming the pathogenic impact of *S. gallinarum* challenge during the acute phase of infection (Table 5).

At day 21 (14 days post-challenge and 7 days after treatment completion), Group C still exhibited ($P < 0.05$) impaired liver functionality and distorted hematological profiles. Notably, Groups B, D, and E showed values similar to the healthy control, indicating that the therapeutic effects of guava leaves extract were sustained for at least 7 days following treatment withdrawal (Table 6).

Table 2. Zone of inhibition (mm) of guava leaves extract and ciprofloxacin against *Salmonella gallinarum*

Concentration / Antibiotic	Zone of Inhibition (mm)
100 mg/mL	16
200 mg/mL	20
300 mg/mL	24
400 mg/mL	27
Ciprofloxacin (5 µg)	16

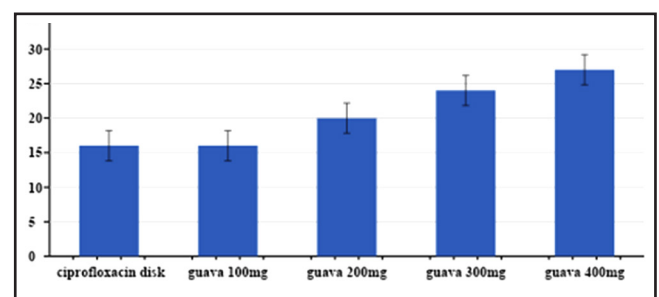


Figure 2. Zone of inhibition (mm) of guava leaves extract at varying concentrations and ciprofloxacin (5 µg) against *Salmonella gallinarum*. Data are presented as mean $\pm$ SD from three independent experiments performed in triplicate ($n = 3$).

Table 3. Minimum inhibitory concentration (MIC) of guava leaves extract against *Salmonella gallinarum* determined by broth microdilution

Well No.	Initial OD ₅₉₅	Final OD ₅₉₅	Difference (Final – Initial)	Concentration (mg/mL)
1	0.657	0.529	–0.128	100
2	0.454	0.312	–0.142	50
3	0.380	0.332	–0.058	25
4	0.388	0.385	–0.003	12.5
5	0.389	0.409	0.020	6.25
6	0.389	0.411	0.022	3.125
7	0.354	0.364	0.010	1.562
8	0.355	0.475	0.120	0.781
9	0.345	0.475	0.130	0.390
10	0.369	0.419	0.050	0.195

Table 4. Serum biochemical and hematological parameters of broiler chickens at day 7 post-treatment

Parameter	Group A (Control)	Group B (Preventive)	SEM	P-value
ALT (U/L)	63.8	61.5	1.47	0.320
ALP (U/L)	19.50	18.25	1.132	0.368
AST (U/L)	13.00	13.50	0.677	0.356
Hb (g/dL)	13.43	14.10	0.477	0.356
RBCs ($\times 10^6/\mu\text{L}$)	4.175	4.375	0.099	0.203
TLC ($\times 10^3/\mu\text{L}$)	4.10	4.15	0.167	0.839

*Note: All P-values > 0.05, indicating no significant difference between groups at this time point.

Table 5. Serum biochemical and hematological parameters of broiler chickens at day 14 post-infection

Parameter	Group A (Control)	Group B (Preventive)	Group C (Infected)	Group D (Treatment)	Group E (Ciprofloxacin)	SEM	P-value
ALT (U/L)	63.0	62.0	167.2	59.5	60.5	3.01	<0.001
ALP (U/L)	10.20	11.25	21.25	10.18	10.65	0.660	<0.001
AST (U/L)	12.25	13.00	58.50	11.12	10.17	0.899	<0.001
Hb (g/dL)	13.47	14.90	9.12	13.47	15.00	0.382	<0.001
RBCs ($\times 10^6/\mu\text{L}$)	4.450	5.500	3.925	4.675	4.900	0.139	<0.001
TLC ($\times 10^3/\mu\text{L}$)	4.075	4.250	4.650	4.100	4.625	0.126	0.004

*Values are expressed as mean $\pm$ SEM. P-values < 0.05 indicate statistically significant differences among groups.

Table 6. Serum biochemical and hematological parameters of broiler chickens at day 21 post-infection

Parameter	Group A (Control)	Group B (Preventive)	Group C (Infected)	Group D (Treatment)	Group E (Ciprofloxacin)	SEM	P-value
ALT (U/L)	65.8	59.3	137.2	62.3	60.0	3.72	<0.003
ALP (U/L)	11.45	9.38	19.00	11.62	10.85	0.909	<0.001
AST (U/L)	13.32	13.70	73.00	12.90	13.12	1.421	<0.004
Hb (g/dL)	12.17	12.87	7.45	13.07	12.15	0.483	<0.001
RBCs ($\times 10^6/\mu\text{L}$)	4.650	4.500	3.375	5.200	4.950	0.2085	<0.001
TLC ($\times 10^3/\mu\text{L}$)	5.200	5.250	3.575	5.975	5.925	0.1688	<0.001

Hematological Assessment

A significant decrease ($P < 0.05$) in Hb, RBC count, and Total Leukocyte Count (TLC) was observed in Group C (Infected) compared to the control and guava-treated groups.

Body Weight Gain, Feed Conversion Ratio, and Mortality

Group C exhibited the lowest body weight gain by day 21 (685 g), whereas Groups B and D demonstrated marginally higher final body weights (855 g and 860 g, respectively) compared to the control Group A (850 g) and the ciprofloxacin-treated Group E (845 g) (Table 7).

Consistent with these findings, the cumulative feed conversion ratio (FCR) at day 21 was poorest in Group C (1.52), indicating reduced feed efficiency due to infection. Group D on the other

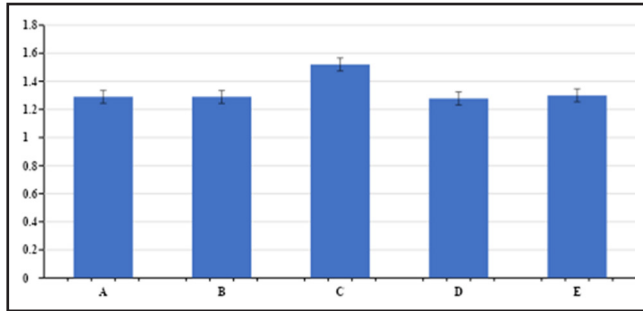
Table 7. Weekly body weight gain (g) of broiler chickens

Group	Day 7	Day 14	Day 21
A (Control)	160	450	850
B (Preventive)	165	445	855
C (Infected)	140	320	685
D (Treatment)	155	455	860
E (Ciprofloxacin)	145	440	845

hand had the best FCR (1.278) followed by Group B (1.289) and was numerically better than the control Group A (1.29) and ciprofloxacin-treated Group E (1.30) (Table 8; Figure 3).

Table 8. Cumulative feed conversion ratio (FCR) of broiler chickens at day 21

Group	FCR
A (Control)	1.29
B (Preventive)	1.289
C (Infected)	1.52
D (Treatment)	1.278
E (Ciprofloxacin)	1.30

**Figure 3.** Cumulative feed conversion ratio (FCR) of broiler chickens at day 21. Data are presented as mean $\pm$ SD (n = 30 birds per group, with three replicates of 10 birds each). Lower values indicate better feed efficiency.

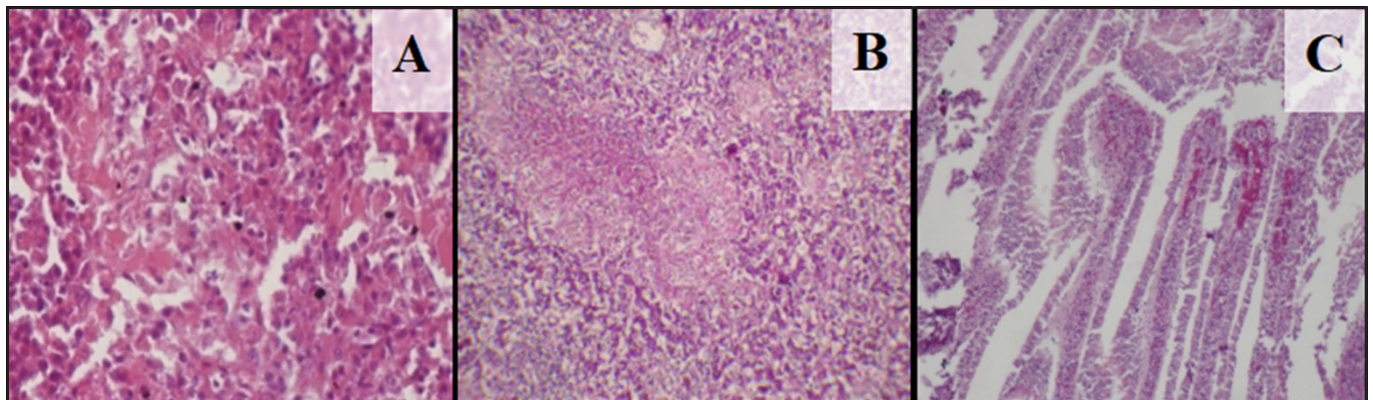
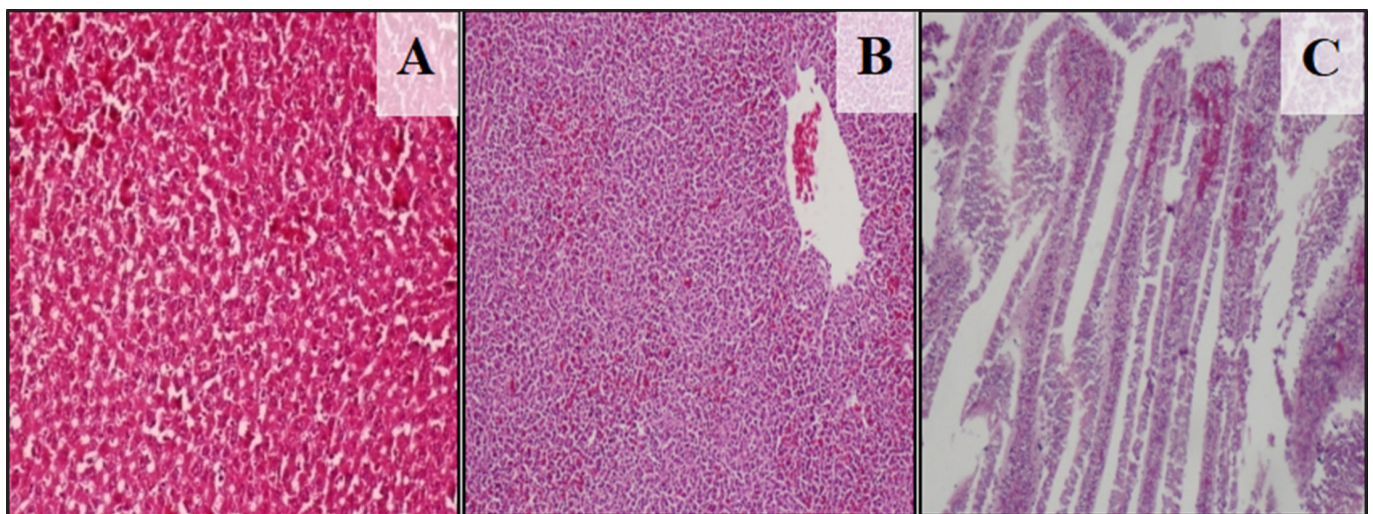
Group C had the highest mortality with 8 deaths (26.7%). Group B (3.3% mortality) and Group D (0% mortality) had significantly lower mortality rates compared to Group E (6.7% mortality) (Table 9).

Histopathological Findings

Histopathological analysis showed that there were specific patterns of tissue change in the experimental groups. Group C (infected, untreated) liver sections showed coagulative necrosis, splenic tissue presented significant necrotic foci, and the small intestine showed mild hemorrhages with inflammatory cells infiltration (Figure 4). Conversely, tissues of Groups B and D (guava extract-supplemented) were found within normal histological ranges, and there was only mild congestion of the intestinal mucosa, which suggested a protective and restorative effect of the extract (Figure 5). Group E (ciprofloxacin-treated): There was mild congestion of the liver, spleen and intestines.

Table 9. Mortality rates of broiler chickens challenged with *Salmonella gallinarum*

Group	Mortality (n)	Mortality (%)
A (Control)	0	0
B (Preventive)	1	3.3
C (Infected)	8	26.7
D (Treatment)	0	0
E (Ciprofloxacin)	2	6.7

**Figure 4.** Photomicrographs of tissue sections from broiler chickens challenged with *Salmonella gallinarum* (Group C, infected untreated). (A) Liver section showing mild degenerative changes in hepatocytes. (B) Liver section exhibiting coagulative necrosis. (C) Small intestine section demonstrating mild hemorrhages in the lamina propria. Hematoxylin and eosin (H&E) stain; magnification as indicated.**Figure 5.** Photomicrographs of tissue sections from broiler chickens treated with guava leaves extract following *Salmonella gallinarum* challenge (Groups B and D). (A) Liver section showing normal hepatic architecture. (B) Spleen section displaying normal splenic parenchyma. (C) Small intestine section with mild congestion. Hematoxylin and eosin (H&E) stain; magnification as indicated.

DISCUSSION

In the current *in vitro* research, preliminary phytochemical screening of the methanolic extract of guava leaves showed the existence of bioactive compounds such as saponins, phenols, tannins, flavonoids and terpenoids. These results agree with the previous ones (Khanna *et al.*, 2025; Biswas *et al.*, 2013). To identify and determine the presence of phenolic compounds, High-Performance Liquid Chromatography (HPLC) was conducted, which revealed that gallic acid was present in the guava leaves extract. This finding is in line with the report of Almutairi *et al.* (2025), who also indicated that guava leaves extract was positive to gallic acid. Moreover, HPLC-DAD characterization of polyphenolic compounds in guava leaves extract revealed that the concentration of phenolic acids and flavonoids was high (Irondi *et al.*, 2016). In particular, the presence of phenolic acids was detected in the form of gallic acid, chlorogenic acid, and caffeic acid, whereas flavonoids were found in the form of rutin, kaempferol, catechin, and quercetin. Gallic acid is detected in the current study, which is close to the results of Irondi *et al.* (2016).

The antibiotic sensitivity of the methanolic guava leaves extract was tested using different concentrations (100 mg/mL, 200 mg/mL, 300 mg/mL and 400 mg/mL) against the standard broad-spectrum antibiotic ciprofloxacin. The findings established that the zone of inhibition was concentration-dependent with the larger the concentration, the larger the inhibition zone. The extract showed concentration-dependent antimicrobial activity on *Salmonella gallinarum*, which is in agreement with the results of Almutairi *et al.* (2025) and Biswas *et al.* (2013). The lowest concentration of guava leaves extract that inhibited *Salmonella gallinarum* was found to be 6.25 mg/mL. This finding is consistent with findings by Olatunde *et al.* (2018), who reported MIC values of 2.5mg/mL to 10mg/mL against Gram-negative and Gram-positive bacteria, respectively, of guava leaves extract.

The *in vivo* study showed that Group B (preventive treatment) and Group D (therapeutic treatment) had significantly lower clinical signs than Group C (infected, untreated). The Group C infected birds had typical signs of fowl typhoid with huddling, ruffled feathers, creamy whitish diarrhea, and body weight loss. These are clinical symptoms that are analogous to those mentioned by Sannat *et al.* (2017). Group E birds that received ciprofloxacin had moderate clinical signs. This result corresponds to the results of Sannat *et al.* (2017), who have found that *Salmonella gallinarum* is intermediate-sensitive to ciprofloxacin.

The liver is a key part of the metabolism in the body and is essential in the elimination of toxic substances (Wang *et al.*, 2023). Serum enzyme levels are regularly done to help in determining physiological functioning and tissue damage. The most important hepatic enzymes include aspartate aminotransferase (AST) and Alanine transaminase (ALT), of which ALT is more localized to the liver, but is also found in muscle, cardiac tissue, pancreas and erythrocytes. Increases in these enzymes are a sign of hepatocellular damage. The extent of ALT and AST increase is different with the underlying etiology and the extent of the hepatic insult.

In this study, assessment of the serum biochemical profile showed that birds in Group C (infected, untreated) had significantly high ($P < 0.05$) levels of ALT, ALP, and AST than the control Group A. This is due to the hepatocellular injury and necrotic lesions in the liver (Kalas *et al.*, 2021). Conversely, Group B (preventive treatment) did not significantly differ ($P > 0.05$) in liver enzyme levels on day 7 with supplementation of guava leaves extract compared to the control Group A. Supplementation of guava leaves extract in Group D (therapeutic treatment) showed significant difference ($P < 0.05$) in AST, ALT, and ALP levels relative to Group C. The results are consistent with those of Uboh *et al.* (2010) who established that oral administration of guava leaves extract in rats did not cause any alteration of ALT, ALP, AST, serum albumin and total protein, which

are indicative of hepatotoxicity. Group E (ciprofloxacin-treated): there was no significant influence on liver enzyme levels.

Hematological examination showed that Group C (acute phase of fowl typhoid) was characterized by a significant reduction ($P < 0.05$) in hemoglobin concentration and red blood cell (RBC) count, which is typically reported during acute phase (Chiroma *et al.*, 2018). Ilesanmi *et al.* (2025) suggest that the anemia caused by acute fowl typhoid is caused by an augmentation in erythrophagocytosis by the reticuloendothelial system. The first major drop in the number of white blood cells (WBC) is probably due to the cytopathic effects of the *Salmonella gallinarum* lipopolysaccharide (LPS) on the circulating leukocytes, observed 2-4 days after infection. This finding is consistent with previous reports by Lam and Munn (2002).

The results on the gain of body weight showed that the growth performance of broilers was improved with the help of the administration of guava leaves extract. This finding is in line with research that indicated that dietary supplementation with 0.2% and 0.5% olive oil had significant ($P < 0.05$) positive effects on final live body weight and daily gain in broiler chicks with no significant effect on the feed intake. In the present research, Groups B and D had slightly more body weight gain on day 21 than the control group.

Group C had the highest mortality (26%), which validated the virulence of the *Salmonella gallinarum* challenge. Birds fed on guava leaves extract (Groups B and D) showed a better fighting ability against the pathogen with only 3 dead. A 6% mortality rate was observed in Group E (ciprofloxacin-treated). Histopathological analysis of Group C showed that there was coagulative necrosis of the liver, necrotic foci in the spleen, and degeneration of intestinal mucosal villi with inflammatory cell infiltration. These pathological observations resemble those of Kumari *et al.* (2013), who indicated the presence of hepatocellular necrosis, focal cellular infiltration, splenomegaly with numerous necrotic foci, and desquamation of the mucosal epithelium leading to villous degeneration and intraluminal necrotic debris. Conversely, groups receiving guava leaves extract-supplemented (B and D) exhibited few histological alterations with only mild congestion in the intestines in the Group E (ciprofloxacin).

This study has several limitations. First, only a single concentration of guava leaf extract (6.25 mg/mL based on MIC) was evaluated *in vivo*; dose-response studies are needed. Second, administration via drinking water, while practical, results in variable intake among individual birds. Third, the extract was not chemically standardized beyond HPLC identification of gallic acid. Fourth, the study duration was limited to 21 days, and no pharmacokinetic studies were conducted. Fifth, direct comparison with ciprofloxacin requires careful interpretation due to different mechanisms of action. Despite these limitations, the findings provide valuable evidence for the antimicrobial potential of guava leaf extract against *S. gallinarum*.

CONCLUSION

This study concludes that guava leaves extract possesses significant antibacterial activity attributable to its polyphenolic constituents. The extract demonstrated a marked inhibitory effect against *Salmonella gallinarum* in broiler chickens, reducing mortality, ameliorating clinical signs, and minimizing histopathological lesions without adverse effects on liver function or body weight gain. While the extract showed promising potential as an alternative antimicrobial agent, direct comparison with antibiotics requires careful interpretation given the different mechanisms of action and dose-response relationships. Further studies with standardized dosing and direct comparative trials are warranted. Nevertheless, based on these findings, guava leaves extract represents a promising candidate for further investigation as a natural therapeutic agent for controlling fowl typhoid.

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Not Applicable.

Conflict of Interest Statement

The authors declare no conflict of interest.

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