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Evaluation of in Vitro and in Vivo Anti-Salmonella Activity of Aqueous Leaf Extract of Solanum Granuloso-Leprosum Against Salmonella Typhi

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ABSTRACT

Typhoid fever caused by *Salmonella typhi* remains major health concern, with increasing antibiotic resistance necessitating alternative therapeutic agents. This study evaluated the *in vitro* and *in vivo* antibacterial , antioxidant and hepatoprotective effects of aqueous leaf extract of *Solanum granuloso leprosum* against *S. typhi* infection. Leaves were macerated in water for 48 hours and screened for phytochemicals.

Antibacterial activity was assessed using agar diffusion method. Forty eight male Wistar rats were divided into six groups: uninfected control, infected untreated, ciprofloxacin-treated (2.5 mg/kg), and infected groups treated orally with extract at 125, 250, and 500 mg/kg for seven days. Bacterial load, serum AST, ALT, ALP, LDH activities, and liver and intestinal SOD, CAT, GSH and MDA were determined. Histopathological examinations were also performed. The extract contained alkaloids, phenols, flavonoids, tannins, and saponins. *In vitro* activity increased with concentration, with 400 mg/ml producing greater inhibition than the standard antibiotic. *In vivo*, extract treatment reduced bacterial load and significantly ($P<0.05$) decreased serum enzyme activities. It increased SOD, CAT, and GSH while reducing MDA levels, with improved liver and intestinal architecture. The aqueous leaf extracts demonstrated antibacterial, antioxidant and hepatoprotective activities, supporting its potential as a therapeutic agent against *S. typhi*-associated complications.

1. INTRODUCTION

Typhoid fever is an enteric infection caused by *Salmonella* enterica serovar *typhi* (*Salmonella typhi*) (Mathur *et al.*, 2012; Bhandari *et al.*, 2024). Symptoms typically develop 6–30 days after infection and include prolonged high fever, weakness, abdominal pain, constipation, headache, vomiting, and occasionally a characteristic rose-coloured rash. Severe cases may cause confusion, while untreated infection can persist for weeks or months (CDC, 2025). Typhoid is transmitted through ingestion of food or water contaminated with faeces from infected individuals and is associated with poor sanitation and limited access to safe drinking water (Tharwani *et al.*, 2022; CDC, 2025). The pathogen can disseminate beyond the intestinal tract to the Peyer's patches, mesenteric lymph nodes, spleen, liver, gall bladder, bone marrow, and bloodstream (Meiring *et al.*, 2023).

Salmonella infections remain a major global public health concern, causing substantial morbidity and mortality (CDC, 2020). Salmonella is widely distributed in the environment and can be recovered from food, water, and animals (Abey *et al.*, 2024). Changes in food production and consumption patterns have contributed to increasing infections (Rincón-Gamboa *et al.*, 2021), while rising antimicrobial resistance has reduced the effectiveness of conventional antibiotic therapy, emphasizing the need for novel antimicrobial agents (Aarestrup *et al.*, 2018).

Solanum granuloso-leprosum Dunal is a small pioneer tree with leaves and fleshy fruits containing small seeds (Knapp *et al.*, 2024). It is distributed across parts of South America, including Argentina, Uruguay, Paraguay, and Brazil, where it occurs mainly in secondary forests and disturbed habitats (Reflora, 2024). The species is known by several vernacular names, including "Cuvitinga," "Fumeiro," and "Capoeira-branca" in Brazil, and "Ambay" in Argentina. In Nigeria, it is locally known as "ewuro ijebu" in Yorubaland and "Yewuru" in parts of Kwara State. In Argentina, *S. granuloso-leprosum* is traditionally used for its anti-inflammatory and calming properties (Petenatti, 1998; Rudke *et al.*, 2019). Its reported traditional medicinal use and potential bioactive constituents suggest that the plant may be a promising source of novel therapeutic agents.

2 Materials and Methods

2.1 Materials

2.1.1 Plant Materials

Fresh leaves of *Solanum granuloso leprosum* were harvested from the premises of late Chief Samuel Ladoke Akintola's compound, Oke-Ado Akintola, Ogbomoso, Oyo State, and were authenticated (LHO 805) sat the herbarium of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria (Plate 1).



Plate 1: Photograph of *Solanum granuloso-leprosum* leaf

2.1.2 Reagents and Chemicals

Solvent (distilled water), Mueller Hinton Agar, Mueller Hinton Broth, and *Salmonella-shigella* agar, were purchased from Fondoz Laboratories Ltd, Nigeria. Assay kits (ALT, AST, ALP, LDH, SOD, Catalase, MDA, and GSH) were purchased from Fortress Diagnostic Limited, United Kingdom. Other reagents used were of analytical grade.

2.1.3 Drug

Ciprofloxacin (500 mg) was procured from Igbala-Olu pharmacy, Ogbomoso, Nigeria and used as the reference antibiotic.

2.1.4 Experimental Animals

Experimental animals consisting of male Wistar albino rats aged 6–8 weeks (150 – 200 g) were purchased from the Animal House of the Department of Physiology, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. The rats were kept under ambient environmental conditions of 25 ± 2 °C, 30–70 % humidity, and a 12 hr light/ 12 hr dark cycle.

2.1.5 Bacteria Isolates

Typed isolates of *Salmonella typhi* (ATCC 14023) were used in this study and were obtained from the Department of Microbiology, Federal University of Technology (FUTA), Akure.

3. Methods

3.1. Preparation of Plant Extract

The leaves of *Solanum granuloso-leprosum* were collected, thoroughly washed under running water to remove sand and debris, and air-dried. The dried leaves were then finely ground into powder with the aid of an electric blender. A 500 g portion of the powdered leaves was soaked in 1.5 L of distilled water for 48 hrs, with manual agitation of the flask using a sterile glass rod after every 24 hr. The resulting mixture was filtered using Whatman filter paper. The filtrate was subsequently lyophilised to obtain a powder extract and was stored till further use (Ogoti *et al*, 2015).

3.2 Qualitative Determination of Phytochemicals

The aqueous leaf extract of *Solanum granuloso-leprosum* was subjected to phytochemical screening for the identification of biologically active secondary metabolites using standard methods described by Harborne (1998) and other established procedures (Chandana and Pathirana, 2024). The major medicinally important phytochemical constituents that were qualitatively analyzed included alkaloids, saponins, tannins, phenols, and flavonoids. These phytochemicals are known for their antimicrobial, antioxidant, anti-inflammatory, and therapeutic properties, which may contribute to the anti-*salmonella* activity of the plant extract against *Salmonella typhi*. All tests were conducted in triplicate to ensure accuracy, reproducibility, and reliability of results.

3.1.3 Inoculum Preparation

Typed isolates of *S. typhi* (ATCC 14023) were obtained from the Postgraduate Microbiology Department, Federal University of Technology (FUTA), Akure. The isolates were confirmed based on cultural, morphological, and biochemical characteristics following standard methods for identifying *Salmonella typhi* (Cheesbrough, 2000). The bacterial strain was grown in nutrient broth for 12–18 hours at 37 °C on a rotary shaker. Cells were grown at 37°C for 18 hours and cultures were kept at 4 °C.

A few colonies of *Salmonella enterica* serovar *typhi (S. typhi*) were transferred with a sterile wire loop from an overnight culture on *Salmonella-shigella* agar (SSA) into sterile Mueller-Hinton Broth (MHB). The culture was incubated at 37 °C for 18 hours. After incubation, the bacterial suspension was centrifuged at 4000 rpm for 10 minutes to separate the broth (supernatant), which was subsequently discarded. The remaining bacterial pellet was washed twice with normal saline, resuspended in normal saline, and adjusted to a concentration of 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/ mL.

3.2 In vitro Anti-Salmonella Activity

3.2.1 Preparation of Mueller Hinton Agar (MHA)

A total of 2.28 g of Mueller-Hinton Agar was dissolved in 60 mL of distilled water. The solution was thoroughly mixed and sterilized in an autoclave. After sterilization, the MHA was allowed to cool slightly before being poured into five sterile Petri dishes, with each dish receiving an equal amount of the medium. The agar was left to solidify before use.

3.2.2 Determination of Zone of Inhibition

A total of 2 g of the extract was dissolved using distilled water to obtain different concentrations (100 mg / mL, 200 mg/ mL, and 400 mg/ mL) of the plant extracts, which were introduced into wells on Mueller Hinton agar plates. The agar well diffusion method as described by Balouiri *et al.* (2016) was utilized to determine the zones of inhibition of bacterial growth. Wells with a diameter of 6 mm were aseptically bored into freshly prepared Mueller-Hinton agar plates. A suspension of *S. typhi* adjusted to 0.5 McFarland standard was used to inoculate the agar. The plates were incubated aerobically at 37 °C and examined after 24 hours. The plates were examined for microbial growth inhibition, and the inhibition zone (IZD) was measured to the nearest millimeter and compared with those produced by the commercial antibiotic ciprofloxacin, which served as the control. Results were recorded for each concentration in triplicate to ensure reproducibility.

3.2.3 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Solanum granuloso-leprosum Extract

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were determined using the broth dilution technique (Anibijuwon and Udeze, 2009). Dilutions of the extract in Mueller Hinton broth were prepared in tubes. The concentration of inoculum was standardized to 0.5 McFarland turbidity. Nine milliliters of Mueller Hinton broth were dispensed into test tubes. 50 µL of each of the standardized bacterial suspension was added, along with 950 µL of the plant extract, respectively. The test tubes containing the different concentration of plant extract 100, 200, and 400 mg/ ml were incubated at 37 °C for 24 hours. The lowest concentration of extract that shows no visible bacterial growth is the MIC. For minimum bactericidal concentration (MBC) determination, the content of the tube that showed no visible bacterial growth were sub-cultured on Mueller Hinton agar and incubated at 37 °C for 24 hours. The lowest concentration of extract for which no colony of bacteria was observed was the minimum bactericidal concentration (MBC).

3.2.5 Determination of In vivo Anti-Salmonella Activity of the Plant Extract

Animals were assigned to appropriate groups for preliminary *in vivo* anti-salmonella screening of *Solanum granuloso-leprosum* based on existing studies indicating that the LD_{50} of *Solanum granuloso-leprosum* exceeds 5000 mg/ kg body weight (Mogana 2020, Adetutu *et al*, 2021). Treatment doses of 125, 250, and 500 mg/ kg body weight of the extract were administered. Forty-eight male rats (150 – 200 g) were used for the study, and the animals were arranged into six groups of eight animals each according to their average body weight. Briefly, rats were allowed to acclimatize for 7 days before the commencement of the experiment. They were allowed free access to food and water throughout the experiment. The rats were fasted overnight and were given 1 mL of saline solution (0.9 % NaCl) containing 1.5×10^8 CFU of *Salmonella typhi* by gavage, except animals in Group 1, which were neither infected nor treated and served as the control group; they received distilled water.

Group 1 received normal feed and water. Group 2 was infected but not treated with the extract or drug. Group 3 was infected and treated with ciprofloxacin (2.5 mg/ kg bw). Group 4, 5, and 6 rats were infected and treated with 125, 250 and 500 mg/ kg body weight of *Solanum granuloso-leprosum* extract respectively for 7 days.

Groups 1–6 of the experimental animals were each administered 1 mL of the prepared bacterial suspension orally using an oral cannula (Tala *et al*., 2015). The establishment of infection was confirmed by measuring the bacterial load of *S. typhi* in the blood of the animals 48 hours post-inoculation. Approximately 50 µL of blood was drawn from the caudal vein of infected animals and diluted in 1 mL of normal saline. This diluted blood sample was then plated on freshly prepared SSA. After a 5-minute assimilation period, the plates were incubated at 37 °C for 18 hours. The presence of clear growth with a black center confirmed *S. typhi* infection (Adeyi *et al*., 2013). Only animals with confirmed infection were included in the experiment. All animals received treatment once daily for seven consecutive days (Day 1 to Day 7 post-infection). Blood

samples were collected on Day 3, Day 5, and Day 7. Samples were cultured on *Salmonella-shigella* agar and incubated for approximately 24 hours for confirmation of *S. typhi* growth

3.2.6 Sample Collection and Preparation

After administration of the final treatment dose, the animals were fasted overnight and then euthanized via cervical dislocation. Blood samples were collected through cardiac puncture into plain tubes for serum preparation. Blood in the plain tubes was allowed to clot before being centrifuged at 4000 × g for 5 minutes. The resulting serum (supernatant) was carefully separated using a Pasteur pipette for biochemical assays, while the remaining pellet was discarded.

The liver and intestine were excised, rinsed with ice-cold normal saline, blotted dry and stored at low temperature to preserve cellular integrity and enzymatic activities for subsequent tissue homogenization and biochemical analyses. Representative portions of each tissue were immediately fixed in 10 % neutral buffered formalin for histological examination to preserve the tissue architecture and prevent post-mortem autolysis.

3.2.7 Biochemical Assays (Liver Function Test)

Liver function test including aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH), were analyzed to assess liver function and tissue damage using spectrophotometric methods according to the protocols provided in the manufacturers' manuals for each assay kit.

3.2.8 Antioxidant Study

The activity of superoxide dismutase (SOD), and catalase (CAT), as well as the concentrations of glutathione (GSH), and malondialdehyde (MDA), were assessed in liver and intestine homogenates. These oxidative markers were determined spectrophotometrically following standard procedures outlined in the manufacturers' manuals for each kit.

3.2.9 Statistical Analysis

Data were presented as mean ± SEM (n = 3 for *in vitro* and n = 4 for animal sample analyses). Statistical analysis was performed using one-way analysis of variance (ANOVA). Differences between means were further analyzed using Duncan's Multiple Range Test (DMRT) at a significance level of $p < 0.05$.

3 RESULTS AND DISCUSSION

3.1 Phytochemical Constituent of the Aqueous Leaf Extract of Solanum granuloso-leprosum

Qualitative phytochemical screening of the aqueous leaf extract of *Solanum granuloso-leprosum* revealed the presence of alkaloids, phenols, flavonoids, tannins, and saponins with varying intensities. Alkaloids and phenols showed strong positive reactions (+++), phenols also gave strong positive responses (+++), tannins were strongly to moderately present (+++/++), flavonoids were moderately detected (++), while saponins showed mild to moderate presence (+/++). The presence of these phytochemicals suggests that the extract contains important bioactive compounds that may contribute to its anti-*salmonella* activity against *Salmonella typhi*.

The strong presence of alkaloids indicates significant antimicrobial potential, as alkaloids are known to inhibit bacterial growth through interference with DNA replication and microbial enzyme activity. Recent studies have reported potent antibacterial activities of alkaloid-rich plant extracts against pathogenic bacteria, including *Salmonella species* (Mehta *et al*., 2021; Orimaye *et al*., 2024)). Phenolic compounds, which were also strongly detected, are recognized for their antioxidant and antimicrobial properties. They help reduce oxidative stress and disrupt bacterial cell membranes, thereby contributing to antibacterial activity and tissue protection during infection (Agyare *et al.*, 2023).

Flavonoids detected in the extract may also contribute to the antibacterial effect through inhibition of microbial metabolism and enhancement of antioxidant defense mechanisms. These compounds are widely known for their antimicrobial and anti-

inflammatory activities (Matotoka *et al.*, 2023). Tannins, which showed strong positivity, possess antimicrobial and antidiarrheal properties and act by precipitating microbial proteins and inhibiting bacterial enzymes (Rahmani *et al.*, 2023). Saponins, though present in lower amounts, may contribute to membrane disruption and immune stimulation, thereby enhancing the overall antibacterial effect of the extract (Ezzat *et al*., 2026).

The phytochemical constituents detected in the aqueous leaf extract of *Solanum granuloso-leprosum* may act synergistically to produce the observed *in vitro* and *in vivo* anti *salmonella* activities against *Salmonella typhi*. These findings support the medicinal relevance of the plant and justify its traditional use in the management of infectious diseases.

3.2 In vitro Anti-Salmonella Activities

The aqueous leaf extract of *Solanum granuloso-leprosum* demonstrated notable antibacterial activity against *Salmonella typhi* in a concentration-dependent manner. The extract exhibited increasing zones of inhibition with increasing concentrations, with the highest activity observed at 400 mg/ ml, producing a zone of inhibition of 23.00 ± 2.65 mm, which was greater than that produced by the standard antibiotic ciprotab (19.67 ± 5.51 mm). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract were 200 mg/ ml and 400 mg/ ml, respectively, indicating strong bactericidal activity.

The pronounced antibacterial activity observed in this study may be attributed to the presence of important phytochemicals such as alkaloids, flavonoids, tannins, phenols, and saponins identified in the extract (Ologun *et al*. 2019). These bioactive compounds have been reported to disrupt bacterial cell walls, inhibit nucleic acid synthesis, and interfere with microbial metabolic pathways. Similar antibacterial activities of medicinal plant extracts against *Salmonella typhi* have been reported by Adetutu *et al.* (2021), who observed significant inhibition of *S. typhi* using *Psidium guajava* and *Azadirachta indica* leaf extracts. Naz *et al*. (2022) also reported that alkaloid-rich plant extracts possess strong antibacterial activity against *Salmonella species*.

The close proximity between the MIC and MBC values suggests that the extract exerts bactericidal rather than bacteriostatic effects. This finding agrees with the report of Mehta *et al*. (2021), who stated that phytochemical-rich medicinal plants can produce irreversible damage to bacterial cells, leading to bacterial death. The higher antibacterial activity observed at increased concentrations further supports the dose-dependent therapeutic potential of the extract.

3.3 Acute Toxicity and Median Lethal Dose (LD_{50}) of Solanum granuloso-leprosum Leaf Aqueous Extract

The acute toxicity study showed no mortality or visible signs of toxicity in rats treated with the aqueous leaf extract of *Solanum granuloso-leprosum* up to 5000 mg/ kg body weight. Therefore, the LD_{50} of the extract was estimated to be greater than 5000 mg/ kg, indicating that the extract is relatively safe.

The absence of toxic effects suggests that the plant extract possesses a wide safety margin and may be suitable for therapeutic use. According to Lorke (1983), substances with LD_{50} values greater than 5000 mg/ kg are considered practically non-toxic. Similar observations were reported by Adetutu *et al*. (2021), who documented the safety of medicinal plant extracts used against *Salmonella typhi*. The low toxicity observed may be associated with the antioxidant phytochemicals present in the extract, which contribute to its protective and pharmacological properties.

3.4 In vivo Anti-Salmonella Activities

3.4.1 Effect of Treatments on Body Weight of S. typhi-Infected Rats

The body weight of untreated *Salmonella typhi*-infected rats decreased significantly when compared with the normal control group, whereas rats treated with graded doses of *Solanum granuloso-leprosum* aqueous leaf extract showed significant improvement in body weight. The increase in body weight observed in the treated groups suggests recovery from infection and improved metabolic function.

Loss of body weight during *Salmonella* infection may be associated with reduced feed intake, dehydration, intestinal disturbance, and increased metabolic stress caused by bacterial infection. Similar observations were reported by Legba *et al.* (2020), who noted that systemic salmonellosis causes weight reduction due to severe physiological stress and tissue damage. The restoration of body weight in extract-treated rats may be linked to the antibacterial and antioxidant properties of the plant extract, which likely enhanced recovery and reduced the severity of infection. Adetutu *et al*. (2021) similarly reported improved physiological conditions and recovery in *S. typhi*-infected animals treated with medicinal plant extracts (Fig. 1).

3.4.2 Bacteremia Progression and Inhibition in Rats Induced with S. typhi and Treated with Solanum granuloso-leprosum Leaf Aqueous Extract

The bacteremia count showed persistent bacterial growth in the untreated infected group throughout the experimental period, whereas treatment with *Solanum granuloso-leprosum* aqueous leaf extract caused a progressive reduction in bacterial load. The highest treatment dose demonstrated the greatest inhibition of bacteremia, indicating significant antibacterial activity *in vivo*.

The persistent bacteremia observed in untreated rats confirms successful establishment of *Salmonella typhi* infection. The reduction in bacterial count following treatment may be attributed to the phytochemical constituents of the extract, particularly alkaloids, flavonoids, and tannins, which possess antimicrobial properties. Similar findings were reported by Adetutu *et al*. (2021), who observed significant inhibition of bacteremia in *S. typhi*-infected rats treated with medicinal plant extracts. Ezzat *et al.* (2026) also reported that plant-derived bioactive compounds enhance immune responses and suppress bacterial proliferation during infection.

The dose-dependent reduction in bacteremia observed in this study further suggests that the extract possesses systemic antibacterial activity capable of limiting bacterial colonization and dissemination within host tissues (Fig. 2).

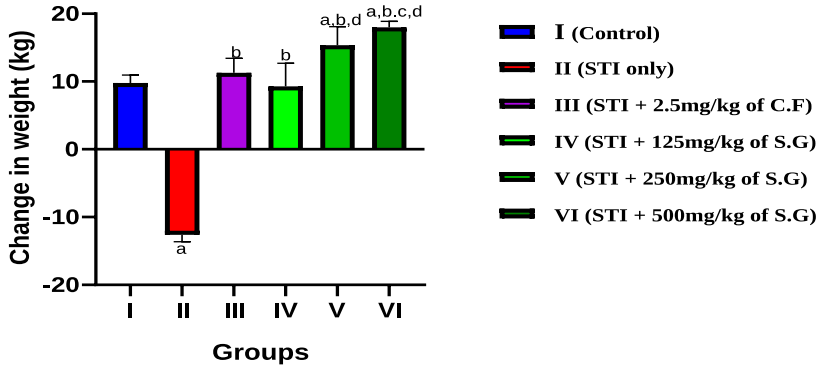


Figure 1: Effect of aqueous leaf extract of *Solanum granuloso-leprosum*on change on body weight of *Salmonella typhi* infected rats

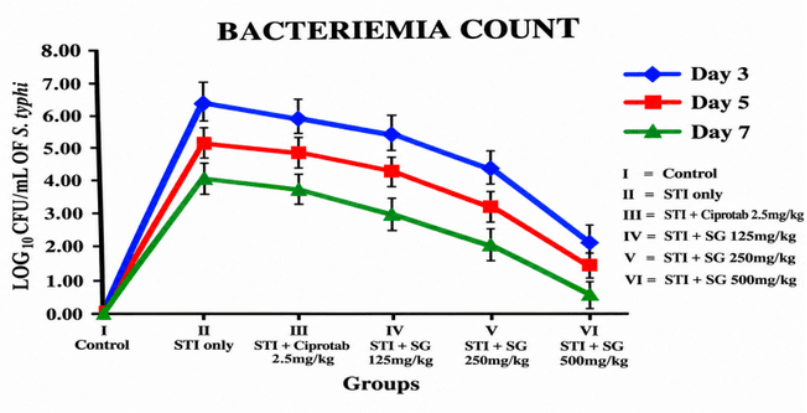


Figure 2: Bacteremia count in *Salmonella typhi*-infected rats treated with *Solanum granuloso-leprosum* after Day 3, 5 and 7

3.5 Liver Function Test

The untreated infected rats showed significant elevations in serum ALT, AST, ALP, and LDH activities when compared with the normal control group. However, treatment with graded doses of *Solanum granuloso-leprosum* aqueous leaf extract significantly reduced the levels of these enzymes toward normal values.

Elevated serum liver enzymes are important indicators of hepatic injury and altered membrane permeability caused by bacterial infection. *Salmonella typhi* infection has been associated with hepatic inflammation, hepatocellular necrosis, and oxidative damage, resulting in leakage of intracellular enzymes into circulation. Similar findings were reported by Iftikhar *et al.* (2024), who observed elevated hepatic biomarkers during systemic salmonellosis.

The reduction in ALT and AST activities following treatment suggests stabilization of hepatocyte membranes and improved liver integrity. Likewise, the decrease in ALP and LDH antivities indicates reduced tissue damage and improved cellular metabolism. These protective effects may be attributed to the antioxidant phytochemicals present in the extract, particularly flavonoids and phenolic compounds, which are known to reduce oxidative stress and inflammation. Rahmani *et al.* (2023) reported that phenolic-rich medicinal plants exert hepatoprotective effects by scavenging reactive oxygen species and preventing lipid peroxidation (Fig. 3).

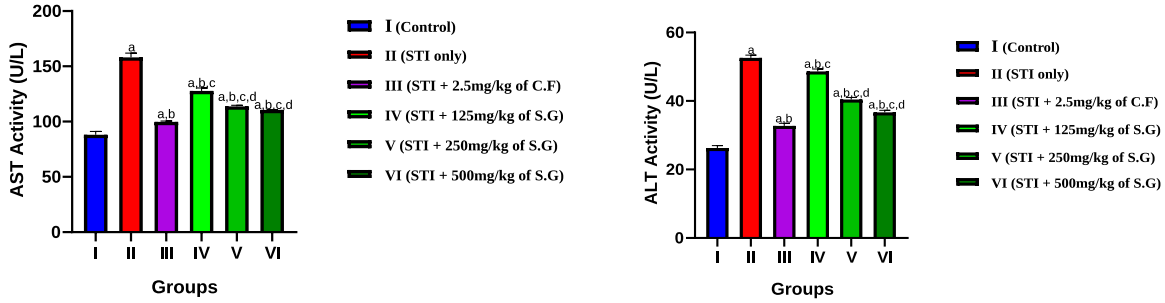


Fig 3: Effect of aqueous leaf extract of *Solanum granuloso-leprosum* on AST and ALT activities in the serum of *Salmonella typhi* infected rats

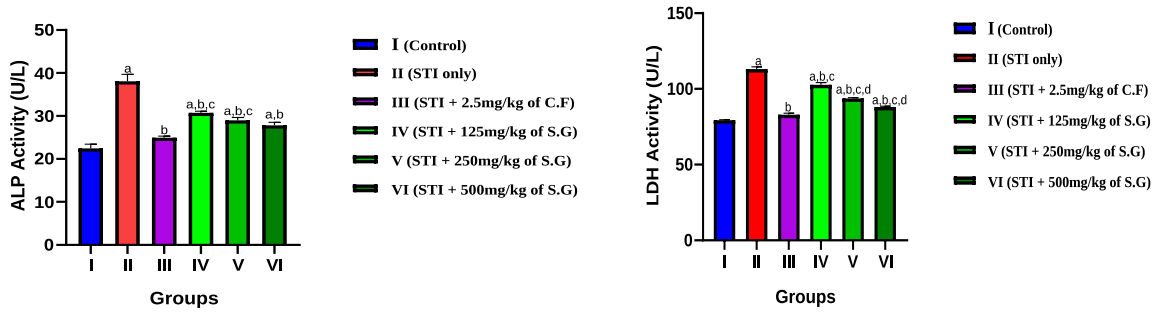


Fig 4: Effect of aqueous leaf extract of *Solanum granuloso-leprosum* on ALT and LDH activities in the serum of *Salmonella typhi* infected rats

3.6 Antioxidant Study

The untreated *S. typhi*-infected rats showed significant reductions in superoxide dismutase (SOD), and catalase (CAT) activities, and reduced glutathione (GSH) concentrarion, with a concomitant increase in malondialdehyde (MDA) concentration in both liver and intestinal tissues. This indicates increased oxidative stress and enhanced lipid peroxidation following *S. typhi* infection. The reduction in antioxidant defenses may result from excessive generation of reactive oxygen species (ROS) during infection, leading to disruption of cellular redox homeostasis and tissue injury. Similar findings have been reported by Abdulgafor *et al.* (2018) and Bizoń *et al.* (2023).

Treatment with graded doses of aqueous *S. granuloso-leprosum* leaf extract improved the antioxidant status of the infected rats, as reflected by increased SOD and CAT activities; and GSH and reduced MDA concentrations. This suggests attenuation of ROS-mediated oxidative damage and inhibition of lipid peroxidation in the liver and intestine. The observed effects may be associated with the antioxidant properties of phytoconstituents such as flavonoids, tannins and phenolic compounds present in the extract, which can scavenge free radicals and support endogenous antioxidant defense mechanisms (Rudrapal *et al.*, 2022).

The findings indicate that aqueous *S. granuloso-leprosum* leaf extract possesses antioxidant and tissue-protective properties against *S. typhi*-induced oxidative injury in the liver and intestine. The reduction in MDA together with modulation of SOD and CAT activities and GSH concentration suggests improved cellular redox balance and reduced oxidative membrane damage, supporting the potential protective role of the extract during *S. typhi* infection (Kouitcheu *et al.*, 2011; Rahmani *et al*., 2023).

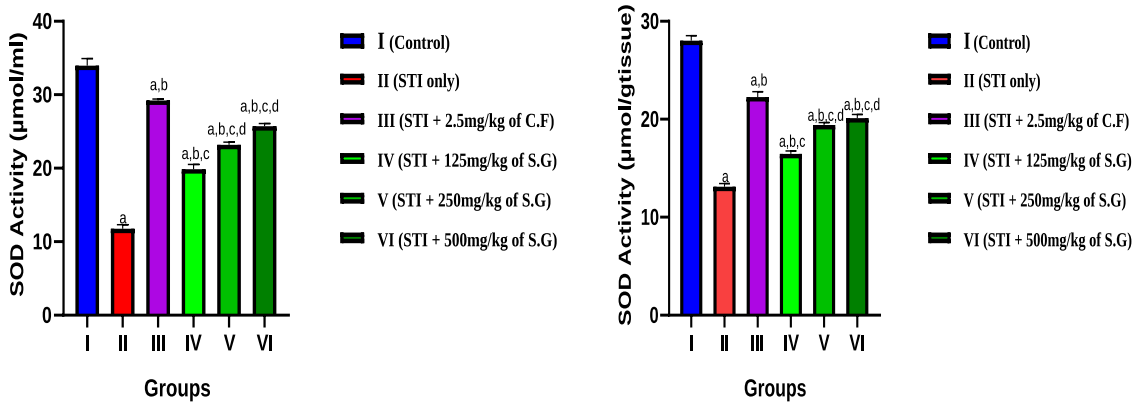


Fig 5: fect of aqueous leaf extract of *Solanum granuloso-leprosum* on SOD activity in the liver and intestine of *Salmonella typhi* infected rats

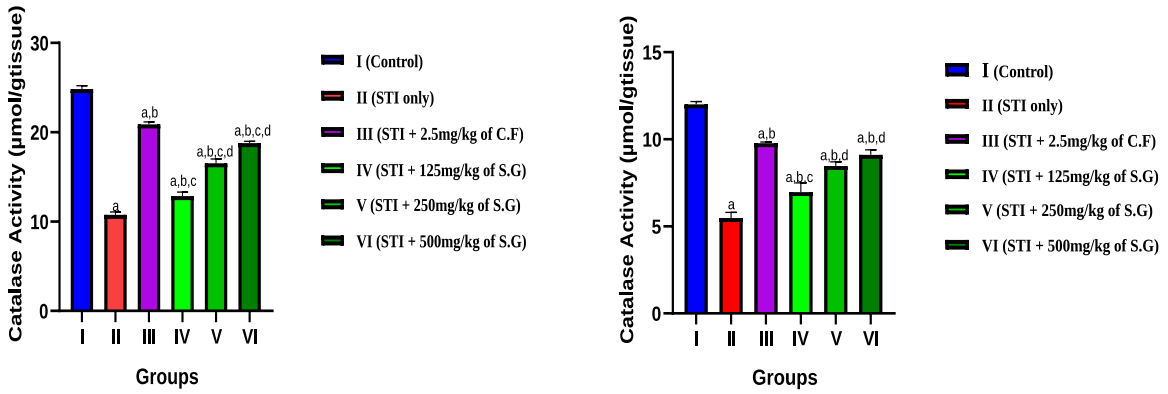


Fig 6: Effect of aqueous leaf extract of *Solanum granuloso-leprosum* on CAT activity in the liver and intestine of *Salmonella typhi* infected rats

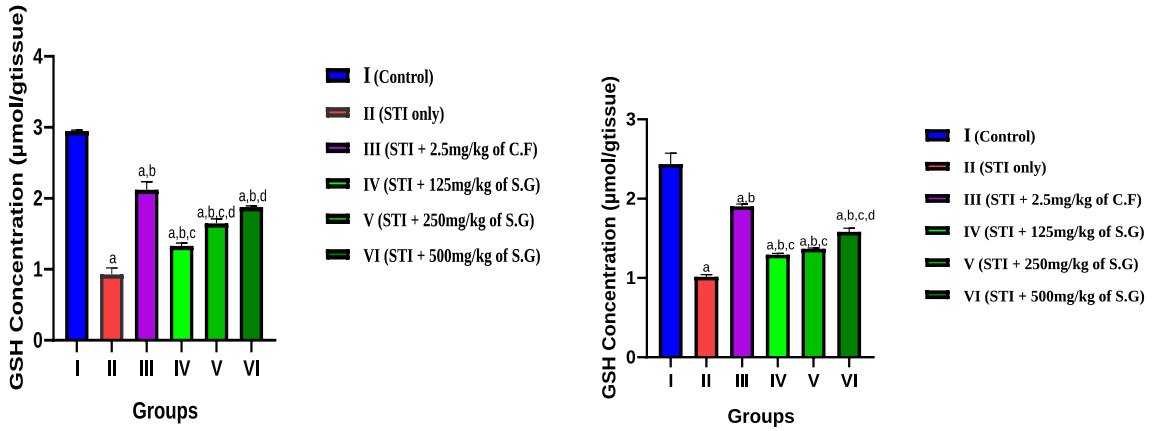


Fig 7: Effect of aqueous leaf extract of *Solanum granuloso-leprosum* on GSH concentration in the liver and intestine of *Salmonella typhi* infected rats

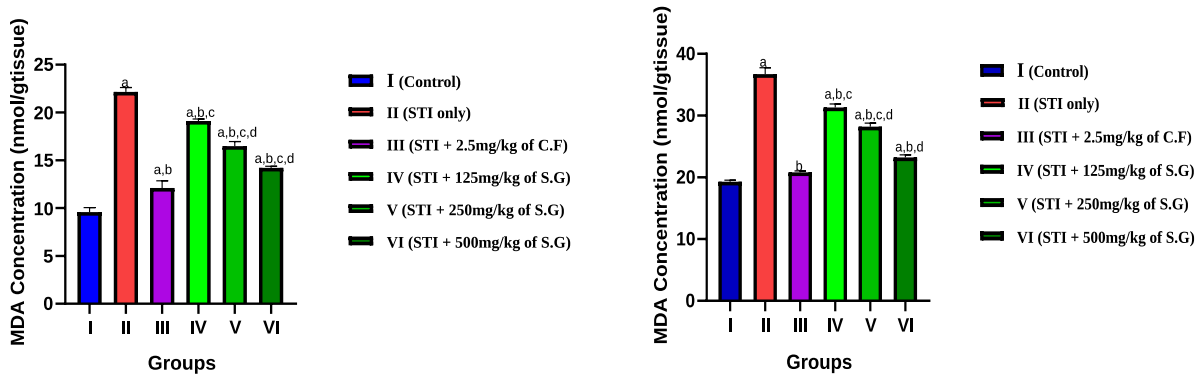


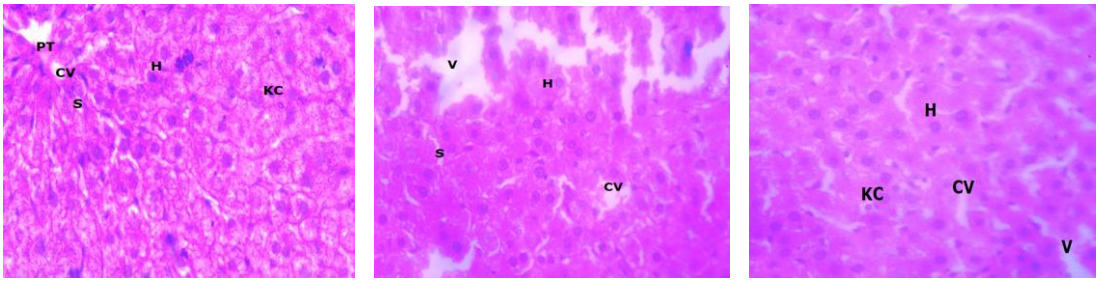
Fig 8: Effect of aqueous leaf extract of *Solanum granuloso-leprosum* on MDA concentration in the liver and intestine of *Salmonella typhi* infected rats

3.7 Liver Histological Study

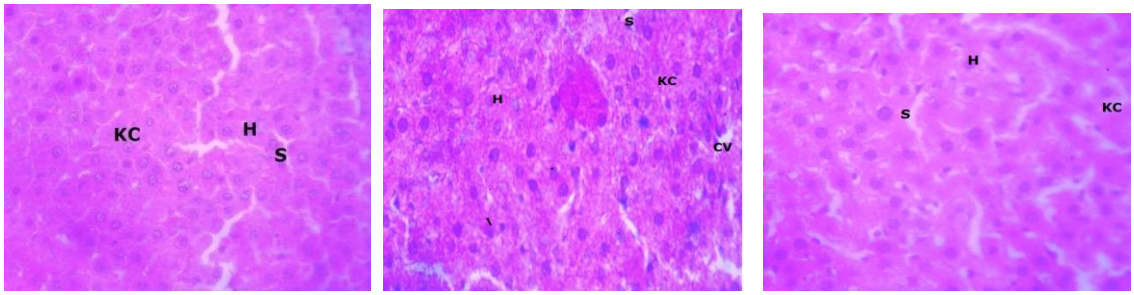
Histological examination showed that *Salmonella typhi* infection caused marked hepatic injury characterized by hepatocellular vacuolation, necrosis, sinusoidal congestion, and inflammatory infiltration in the untreated infected group. These lesions indicate severe hepatocellular damage resulting from bacterial toxins, oxidative stress, and inflammatory responses.

Treatment with *Solanum granuloso-leprosum* leaf extract improved liver architecture in a dose-dependent manner by reducing vacuolation, necrosis, and inflammatory changes while preserving hepatocyte arrangement and sinusoidal integrity. The higher extract dose showed greater hepatoprotection, with histological features approaching those observed in the Ciprofloxacin-treated group.

The protective effect of the extract may be attributed to its phytochemical constituents, including flavonoids, phenolics, alkaloids, and terpenoids, which possess antioxidant, antimicrobial, and anti-inflammatory activities. The findings suggest that *S. granuloso-leprosum* leaf extract effectively attenuates *S. typhi*-induced hepatic damage and supports restoration of normal liver histology (Plate 2).



a: Mag X100, (H&E) **b: Mag X100, (H&E)** **c: Mag X100, (H&E)**



d: Mag X100, (H&E) **e: Mag X100, (H&E)** **f: Mag X100, (H&E)**

Plate 2: Photomicrographs of Liver Section of Rats Induced With *S. typh*i and Treated With Different Doses of Aqueous Leaf Extract of *Solanum granuloso-leprosum*

(a.) Control (b.) *S. typhi* only (c.) *S. typhi* + 2.5mg/kg bw CF (d.) *S. typhi* + 125 mg/kg bw SG (e.) *S. typhi* + 250 mg/ kg bw SG (f.) *S. typhi* + 500 mg/ kg bw SG

SG = *Solanum granuloso-leprosum*

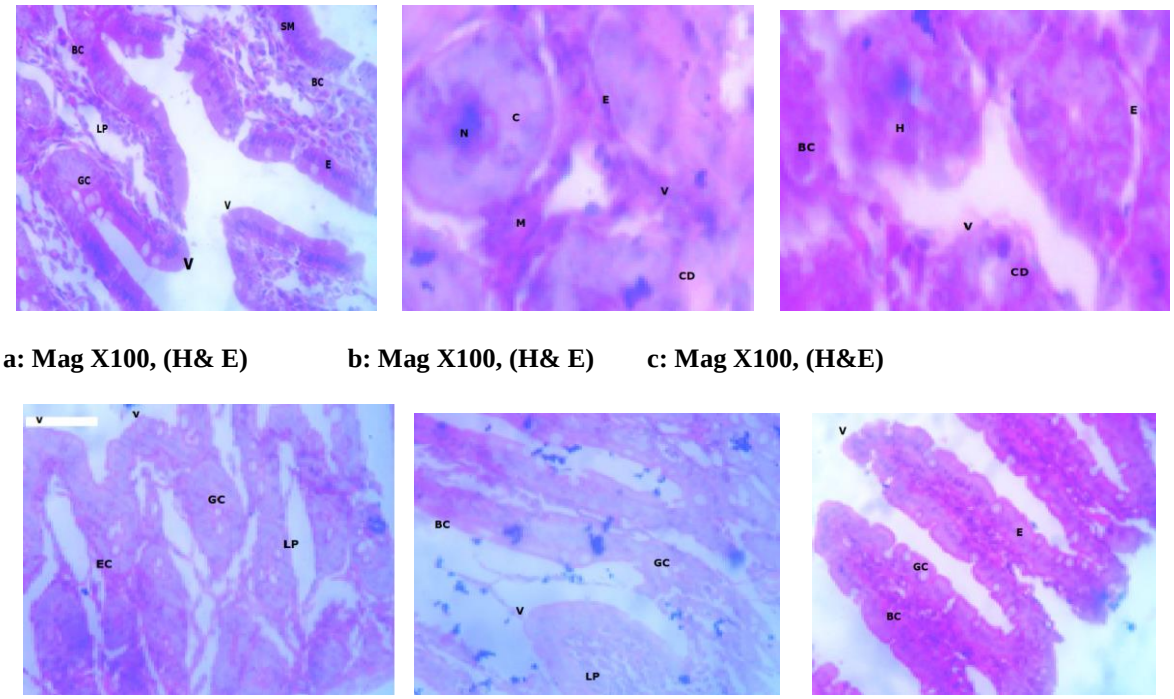
CF = Ciprofloxacin

3.8 Intestine (Ileum) Histological Study

The ileum of untreated infected rats showed severe mucosal injury characterized by villous distortion, goblet cell depletion, crypt abnormalities, epithelial degeneration, and dense inflammatory infiltration, indicating significant intestinal damage caused by *Salmonella typhi* infection.

Administration of *Solanum granuloso-leprosum* leaf extract produced dose-dependent improvement in intestinal morphology, with better preservation of villous architecture, reduced inflammatory infiltration, and improved epithelial integrity observed in treated groups. The highest extract dose demonstrated the greatest mucosal protection, comparable to the ciprofloxacin-treated group.

These protective effects may be linked to the antimicrobial, antioxidant, and anti-inflammatory properties of the plant phytochemicals, which help reduce bacterial-induced oxidative stress and mucosal injury. The findings support the potential of *S. granuloso-leprosum* leaf extract in protecting intestinal tissue during *S. typhi* infection (Plate 3).



a: Mag X100, (H& E) **b: Mag X100, (H& E)** **c: Mag X100, (H&E)**

d: Mag X100, (H&E) **e: Mag X100, (H&E)** **f: Mag X100, (H&E)**

Plate 3: Photomicrographs of the Intestine Section of Rats Induced With *S. typhi* and Treated With Different Doses of Aqueous Leaf Extract of *Solanum granuloso-leprosum*

(a.) Control (b.) *S. typhi* only (c.) *S. typhi* + 2.5mg/kg bw CF (d.) *S. typhi* + 125 mg/kg bw SG (e.) *S. typhi* + 250 mg/ kg bw SG (f.) *S. typhi* + 500 mg/ kg bw SG

SG = *Solanum granuloso-leprosum*

CF = Ciprofloxacin

3.9 Discussions

The present study evaluated the *in vitro* and *in vivo* anti-*Salmonella* activities of the aqueous leaf extract of *Solanum granuloso-leprosum* against *Salmonella typhi*, with emphasis on its phytochemical constituents, antibacterial efficacy, safety profile, biochemical effects, and antioxidant properties. The findings obtained from this study demonstrate that the plant extract possesses significant antibacterial, antioxidant, and organ-protective activities, thereby supporting its ethnomedicinal relevance in the management of salmonellosis.

Phytochemical screening revealed the presence of important bioactive compounds including alkaloids, flavonoids, tannins, saponins, and phenols. These phytochemicals are widely recognized for their antimicrobial and antioxidant activities. Alkaloids have been reported to inhibit bacterial growth through interference with DNA replication and protein synthesis,

while flavonoids and phenolic compounds function as potent free radical scavengers and membrane stabilizers (Naz *et al*., 2022; Zhang *et al*., 2023). Tannins and saponins also contribute to antimicrobial activity by disrupting microbial cell membranes and inhibiting bacterial enzymes. The abundance of these secondary metabolites in the extract may therefore explain the observed anti-Salmonella activity in both *in vitro* and *in vivo* experiments.

The acute toxicity study demonstrated no mortality or visible signs of toxicity in experimental rats treated with the extract up to 5000 mg/kg body weight, indicating that the LD_{50} is greater than 5000 mg/kg. This suggests that the extract is relatively safe and possesses a wide therapeutic margin. According to Lorke (1983), substances with LD_{50} values above 5000 mg/kg are considered practically non-toxic. Similar observations have been reported for medicinal plant extracts used in the treatment of infectious diseases, including *Salmonella typhi* infection (Adetutu *et al*., 2021). The safety profile observed in this study further supports the possible therapeutic application of *Solanum granuloso-leprosum*.

The *in vitro* antibacterial study showed that the extract exhibited dose-dependent inhibitory activity against *Salmonella typhi*, with the highest concentration producing a greater zone of inhibition than the standard antibiotic. In addition, the close relationship between the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) suggests that the extract possesses bactericidal activity. This finding indicates that the extract is capable of directly eliminating bacterial cells rather than merely suppressing their growth. Similar reports by Mehta *et al.* (2021) and Adetutu *et al*. (2021) demonstrated that medicinal plants rich in alkaloids and flavonoids possess significant bactericidal activity against *Salmonella species*. The antibacterial activity observed in this study may therefore be attributed to synergistic interactions among the phytochemical constituents of the extract.

The *in vivo* study further demonstrated that treatment with the aqueous leaf extract significantly improved the physiological condition of *Salmonella typhi*-infected rats. Untreated infected animals exhibited persistent bacteriemia and progressive loss of body weight, indicating severe systemic infection and metabolic stress. However, extract-treated groups showed marked reduction in bacterial load and significant improvement in body weight. The reduction in bacteriemia suggests that the extract possesses systemic antibacterial activity capable of limiting bacterial proliferation within host tissues. Similar findings were reported by Adetutu *et al.* (2021), who observed reduced bacteriemia and improved recovery in Salmonella-infected animals treated with medicinal plant extracts.

Biochemical analysis revealed significant elevations in serum ALT, AST, ALP, and LDH activities in untreated infected rats, indicating hepatic injury and altered membrane permeability induced by *Salmonella typhi* infection. Elevated liver enzymes are commonly associated with hepatocellular damage resulting from oxidative stress and inflammatory responses during bacterial infection. Treatment with *Solanum granuloso-leprosum* extract significantly reduced the activities of these enzymes toward normal levels, suggesting hepatoprotective effects of the extract. The restoration of liver enzyme activities may be linked to the antioxidant and membrane-stabilizing properties of flavonoids and phenolic compounds present in the extract. Similar observations were reported by Iftikhar *et al*. (2024), who noted that medicinal plant extracts reduced hepatic damage associated with systemic bacterial infections.

Oxidative stress markers evaluated in both liver and intestinal tissues showed that untreated infected rats experienced significant reductions in antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), alongside increased malondialdehyde (MDA) levels. These findings indicate severe oxidative stress and enhanced lipid peroxidation caused by *Salmonella typhi* infection. Reactive oxygen species generated during infection can overwhelm endogenous antioxidant defenses, leading to membrane damage and tissue injury. Similar findings were reported by Abdulgafor *et al.* (2018), who associated *Salmonella*-induced oxidative stress with depletion of antioxidant enzymes and elevated lipid peroxidation.

Treatment with the extract significantly restored SOD, CAT, and GSH levels while reducing MDA concentrations in both liver and intestinal tissues. The improvement in antioxidant status suggests that the extract enhanced endogenous antioxidant defense systems and reduced oxidative damage. Flavonoids, phenols, and tannins present in the extract may have contributed to these protective effects through free radical scavenging and inhibition of lipid peroxidation. Rahmani

et al. (2023) similarly reported that phenolic-rich medicinal plants effectively attenuate oxidative stress and preserve tissue integrity during microbial infections.

The histopathological findings further supported the biochemical and oxidative stress results. Untreated infected rats showed varying degrees of hepatic and intestinal tissue damage characterized by inflammation, cellular degeneration, and necrosis. In contrast, extract-treated groups exhibited improved tissue architecture with reduced inflammatory infiltration and enhanced cellular recovery. These observations confirm the protective effects of the extract against Salmonella-induced tissue injury.

The findings of this study demonstrate that the aqueous leaf extract of *Solanum granuloso-leprosum* possesses significant antibacterial, antioxidant, hepatoprotective, and intestinal protective properties against *Salmonella typhi* infection. The observed therapeutic effects are likely mediated through the combined activities of its phytochemical constituents, which act synergistically to suppress bacterial proliferation, reduce oxidative stress, and preserve tissue integrity. These findings provide scientific support for the traditional use of the plant in the management of infectious diseases and highlight its potential for development into a plant-based anti-*Salmonella* therapeutic agent.

4. Conclusions

This study provides substantial evidence that the aqueous leaf extract of *Solanum granuloso-leprosum* possesses significant *in vitro* and *in vivo* anti-*Salmonella* activity against *Salmonella typhi* infection. This study provides scientific evidence thereby supporting the traditional use of *Solanum granuloso-leprosum* in the treatment of infectious diseases, particularly salmonellosis. The research established that the aqueous leaf extract possesses potent antibacterial, antioxidant, hepatoprotective, and intestinal protective properties against *Salmonella typhi*. The findings contribute to the growing search for safer and effective plant-derived alternatives to conventional antibiotics in combating antibiotic-resistant typhoid infections. Furthermore, the study highlights the therapeutic potential of the plant for future pharmaceutical development and supports global efforts toward the discovery of affordable natural antimicrobial agents.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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