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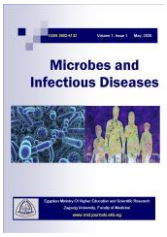

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## Original article

# Anti-*Salmonella* activity of *Gossypium hirsutum* leaf extracted with carbonated drink and its toxicological evaluation *in-vivo*

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**ABSTRACT**

**Background:** Although antibiotics are used to treat typhoid fever, there is a need to look for alternative treatment methods because *Salmonella typhi (S. typhi)* has become aggressively resistant to standard antibiotic treatments. **Methods:** This experimental study used the carbonated beverage "7UP" as the extraction solvent to examine the anti-*Salmonella* activity of *Gossypium hirsutum* leaf extract on *Salmonella typhi*. Extraction of bioactive components of the plant leaf, *in-vitro* and *in-vivo* anti-*Salmonella* activity of the extract was carried out using essential microbiological tools. **Results**: *In vitro,* anti-*Salmonella typhi* study showed that ciprofloxacin had the highest zone of inhibition 36.71±0.31 and 36.14±0.10mm against typed (ATCC 14028) and clinical isolates, respectively, while the zones of inhibition of the extract at different concentrations against *Salmonella typhi* were zero, however, the diameter of zone of inhibition (ZOI) of section at 800 mg/ml was 1.42±0.33 and 1.21±0.01mm against clinical and typed isolates respectively. At larger (3500 mg/ml/kg) and lower (10 mg/ml/kg) doses, there was no death in experimental rats during the acute toxicity assessment. After three days of therapy, the extract reduced salmonella fecal shedding from 71.14±0.31 to 6.02±0.17 × $10^3$ Cfu/g and guaranteed 100% survival against experimental salmonellosis. Different functional groups in the extract, including alkene, anhydride, alcohol, sulfate, nitro compounds, alkanes, and a carboxylic acid, were observed. **Conclusions**: The use of '7UP' as an extraction solvent for medicinal plants in our community for treating diverse medical ailments, especially infectious diseases, is justified. However, it could pose a toxicological effect.

## Introduction

*Salmonella enterica* subspecies *enterica* serovar *typhi* is the causative agent of typhoid fever, an enteric fever [1]. The abrupt onset of a persistent, systemic temperature and excruciating headaches, nausea, and appetite loss are symptoms of this illness [1]. Other signs and symptoms include diarrhea or constipation, spleen enlargement, the potential for meningitis, and general malaise [1, 2].

In many low- and middle-income countries, *Salmonella enterica* is the most common cause of community-acquired bloodstream infections [3,4]. Typhi, Paratyphi A, Paratyphi B,

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and Paratyphi C are serovars of *Salmonella enterica* that can be collectively referred to as "typhoidal Salmonella." In contrast, other serovars are categorized as "non-typhoidal *Salmonella*" (NTS) [4]. Typhoid and paratyphoid fevers, collectively known as enteric fever, are brought on by typhoidal Salmonella strains, which can only infect humans [4]. Non-typhoidal *Salmonella* strains can either be host generalists, colonizing or infecting a wide variety of vertebrate animals, or they can be specialized or limited to a specific non-human animal species [5].

Approximately 21 million infections and 222,000 fatalities are brought on *by Salmonella typhi* (*S.typhi*) each year globally [6]. The antibiotics of choice for treating enteric fever include ampicillin, chloramphenicol, cotrimoxazole, fluoroquinolones, and third-generation cephalosporins. However, typhoidal *Salmonella* species in developing nations are becoming resistant to standard antibiotics, including ampicillin, chloramphenicol, cotrimoxazole, and fluoroquinolones [7]. If enteric fever is incorrectly diagnosed, used, and treated with antibiotics, the fatality rate is predicted to rise by 30% [8]. Blood cultures are still the most reliable way to diagnose enteric fever. Biochemical assays identify the organisms further once it has been isolated from a culture assay [9]. Many healthcare settings frequently use agglutination tests and immunoassay methods [10]. However, these serological techniques are ineffective due to their limited sensitivity and specificity [11]. Obtaining *S. typhi* from clinical samples, including urine, bone marrow, rose spot extracts, duodenal aspirates, and stool, is required to diagnose enteric fever [12]. Native American and African indigenous cultures used plants in their healing rituals, while other cultures created orthodox medicines that included herbal remedies [13]. Researchers discovered that people frequently used the same or related plants for the same objectives [13].

It is necessary to look for new treatment techniques when *S. typhi's* resistance to standard antibiotic treatments increases [14]. Additionally, several studies have demonstrated the effectiveness of *Salmonella*-fighting plant extracts. In earlier research [15,16], the natural active ingredient in the plant was extracted using several organic solvents; however, there is little to no information on the solvent used locally for the extraction of bioactive compounds in medicinal plants. By evaluating its inhibitory growth potential on *S. typhi* in-vivo and using the fizzy drink brand "7UP" as an extraction solvent for medicinal plants, this study focuses on the effectiveness of the conventional practice of using it to treat typhoid fever both *in-vitro* and *in-vivo*.

This investigation examined the anti-Salmonella properties of *Gossypium hirsutum* (*G. hirsutum*) leaves harvested using the fizzy beverage brand "7UP." We sought to determine the anti-Salmonella activity of *G. hirsutum* leaves carbonated drink extract in vitro, determine the anti-*Salmonella* activity of *G. hirsutum* leaves carbonated drink extract in vivo using Wistar Albino rats, and determine the chemical composition of *G. hirsutum* leaves carbonated drink extract using Fourier Transforms Infrared (FTIR) spectroscopy. *Gossypium hirsutum*, also known as upland cotton or Mexican cotton, is the most widely planted species of cotton in the world. Globally, about 90% of all cotton production is of cultivars derived from this species.it is an important information about this plant.

## Materials and Methods

### Collection of leaves of *Gossypium hirsutum*

Fresh *G. hirsutum* leaves were collected from the North gate of the Federal University of Technology in Akure, Ondo State, before sunrise to Prevent Photo-oxidation. The leaves that had neither damage nor chlorosis were separated and kept clean for further work before being identified by the Department of Crop, Soil and Pest Management expert. In the Southwestern region of Nigeria, Yoruba people commonly refer to the cotton plant as "ewe owu."

### Preparation of plant extract

The technique of **Benamar et al.** [17] applied a bit of slight modification. *Gossypium hirsutum* shoots (100 days old) were washed thrice with tap water to remove dust particles and further rinsed with distilled water to avoid any other contaminants. Cleaned shoots (10 g) were cut into small pieces and ground into a rough paste. To this, the "7Up" drink was added in a 250 ml Erlenmeyer flask, followed by heating at 80 °C for 30 min. The extract was then cooled to room temperature (RT), centrifuged at 8000 × g for 5 min at RT and filtered using Whatman no. 1 filter paper to afford a clear solution. The CSE obtained was stored at 4 °C for further use. After 72 hours, it was sieved using a muslin cloth and filtered using Millipore filter paper. The filtrate rinsed with

distilled water to avoid (Union Laboratories England). The extracts were preserved in a sterile bottle at 4 °C ready for use [18, 19].

**Test organisms**

The Federal University of Technology Akure's Department of Microbiology provided the clinical bacterial strains. *S. typhi* from a clinical isolate and *S.typhi* of type (ATCC 14028) were used as controls. Following accepted procedures for identifying *S. typhi*, the isolates were confirmed based on cultural, morphological, and biochemical characteristics [20]. The bacterial strain was cultivated in nutrient broth on a rotary shaker for 12 to 18 hours at 37 °C. Cultures were retained at 4 °C while cells were grown at 37 °C for 18 hours.

***In-vitro* antimicrobial susceptibility tests**

Standardization of the inoculum

The inoculum was prepared by inoculating colonies of new test cultures into sterile distilled water. The turbidity was compared to 0.5McFarland standard prepared according to the method of Cheesbough [20].

**Antibiotics susceptibility test using commercial antibiotics**

As described by Cheesbough, the disc diffusion method [20] was used to determine the antibiotic sensitivity test results for the bacterial isolates. Using sterile swabs, a standard inoculum of 18 hours' worth of soup was applied in triplicates to Muller Hinton agar (MHA). On the dish, the antibiotic discs were distributed evenly. The diameter of the inhibitory zone was then measured and recorded after the plates had been incubated for 24 hours at 37 oC. The commercial antibiotics discs (Fondoz Laboratories Ltd, Nigeria) used were chloramphenicol (CH) 30 µg, sparfloxacin (SP) 25 µg, ciprofloxacin (CPX) 10 µg, amoxicillin (AM) 25 µg, augmentin (AU) 30 µg, gentamycin (CN) 10 µg, pefloxacin (PEF) 5 µg, tetracycline (TET) 5 µg, streptomycin (S) 10 µg and septrin (SXT) 30 µg.

**Antibiotics susceptibility test of *G. hirsutum* leaf extract**

Dimethylsulphoxide (DMSO), 50% v/v, was used to dissolve and dilute the extracts to provide a range of concentrations (400, 500, 700, and 800 mg) in 0.1 mL. The MHA plate's agar wells were filled with extracts of *G. hirsutum* leaves at concentrations of 400 mg/ml, 500 mg/ml, 700 mg/ml, and 800 mg/ml. After 24 hours of aerobic incubation at 37 oC, the plates were inspected. The plates were tested for microbial growth inhibition, and the inhibition zone diameter (IZD), which served as a control, was measured to the nearest millimeter and contrasted with those results. Additionally, an isolate of *Salmonella* was cultivated and treated with various quantities of the extract to test its effects on the anti-*Salmonella* efficacy of the extract in broth of extract inside the test tube and incubated at 37 oC for 24 hours after which 100 µl was pour plated and number of colonies was counted after incubation period [21].

**Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *G. hirsutum* extracts**

Using the broth (tube) dilution method, the extracts' MIC and MBC were calculated [22]. In tubes, extract dilutions in Mueller Hinton broth were made. The Mueller Hinton broth in tubes holding the various concentrations of plant extract, 400 mg/ml, 500 mg/ml, 700 mg/ml, and 800 mg/ml, were then inoculated with 0.5 ml of the standardized culture. The inoculum concentration was likewise standardized to 0.5 McFarland's turbidity. After that, the tubes were incubated for 24 hours at 37 °C. Values for MIC and MBC were noted.

**Preparation of standard inoculums for *in-vivo* assay**

**Chukwuka et al.** [23] procedure was utilized to prepare the clinical isolates for in vivo use in the in vitro assay. Colonies that had grown overnight were transferred to a sterile saline tube. On a piece of white paper with black lines, the bacterial suspension was compared to the 0.5 McFarland standards. By including sterile saline or additional bacterial growth, the bacterial solution was adjusted to the right density as the 0.5 McFarland. The infectious dose of Salmonella, 1.5×108 Cfu/ml, was then obtained by diluting the bacterial suspension.

**Source of experimental Wistar albino rats**

The Department of Microbiology, School of Life Sciences, Federal University of Technology, Akure, provided the experimental rats. They were maintained on standard feed (vital feed) and water while being acclimated for 14 days in a wooden cage under typical environmental conditions with a 12-hour light/dark cycle. According to **Zimmermann's** [24] documentation, the laboratory animals were used in compliance with laboratory practice regulations and the idea of ethical laboratory animal care. Both sexes of them weighed between 50 and 91 g.

**Acute toxicity test**

The acute toxicity test was conducted using **Lorke's** [25] methodology. There were two phases to the study's execution. There were six groups of three rats, each comprising 18 animals. Various extract doses were given to each group. They were kept under observation for 24 hours, 48 hours, and 72 hours to observe the rats' behavior and determine whether any death occurred, and 72 hours. While the other three received higher doses of the extract (1500, 2500, and 3500 mg/kg body weight), three groups received lesser doses of oil (10, 100, and 1000 mg/kg body weight) dose.

Then the LD50 is calculated by the formula:

$LD50 = \sqrt{(D0 \times D100}$

D0= Highest dose that gave no sign of toxicity

D100= Lowest dose that produced signs of toxicity

***In-vivo* anti-*Salmonella* assay**

For this investigation, Wistar albino rats were utilized. They were separated into 5 groups of 3 animals each, with both sexes present in each group, and each group weighing between 57 and 91 g. Salmonellosis was induced using a modified version of the **Pan et al.** [26] technique. Except for the animals in group 5, the rats were administered 1 mL of saline solution (0.9% NaCl) containing 1.5×108 Cfu of Salmonella typhi after being starved for the previous night (which were neither infected nor treated and used as neutral control; they received distilled water). Groups from group 4 (infected but untreated) were given distilled water during the treatment period, and as negative control groups, animals from group 3 (treated with ciprofloxacin) served as positive control groups. Group 2 was not infected but was fed with extract to monitor the effect of the extract alone. In contrast, group 1 was infected and treated with LD50 extract concentration in mg/ml/body weight (kg) by gastric intubation for 3 consecutive days. A consistent increase in the bacterial load over the course of the subsequent four days indicated the formation of the infection, which was confirmed by measuring the bacterial load in the animal feces one day before infection and throughout that same period. The animals were kept at room temperature with a 12-hour light/dark cycle and were given access to ad libitum regular animal feed and water. Fecal samples were taken every day after the bacterial suspension (inoculum) had been administered, and the number of bacteria per gram of feces (Cfu/g) was calculated. In actuality, the feces were mixed with saline distilled water (0.9% NaCl) at a ratio of 1 g to 2 mL of suspension. Aliquots (100 µL) of fecal suspensions were serially diluted in saline distilled water (0.9% NaCl) and then plated on duplicate Salmonella-Shigella agar plates, which were subsequently incubated overnight at 37 ºC.

**Collection of blood samples**

The approach taken by **Gatsing et al.** [27] was followed with little alteration. Rats were anesthetized with chloroform vapor after the treatment period so they could be dissected. Utilizing a cardiac puncture, blood samples were taken and placed in two distinct tubes, one of which included an anticoagulant, ethylene diamine **tetraacetic acid (EDTA).**

**Measurement of the percentage weight gain**

The weights of the rats were taken daily using a digital weighing balance. The mice were demobilized and fixed in a container while taking note of the weight of the container. The weight increase or weight loss of animals was evaluated, and the percentage of weight gain was determined [28].

**Temperature evaluation**

The temperatures of the rats were checked daily using a clinical thermometer fixed into their anus for 30 seconds after demobilizing them in a fixed container. The readings were taken daily for the period of the experiment.

**Histopathological analysis**

**Di Fiore** [29] traditional methods were used to create and examine tissue cross-sections. Small portions of liver were taken from the animals, fixed in 10% formalin, dehydrated in increasing alcohol concentrations, and then cleaned in xylene. The fixed tissue was sectioned into five micrometer-thick sections using a rotary microtome, embedded in paraffin wax, and stained with hematoxylin and eosin. After that, the slices were viewed under a light microscope and captured on camera using a tiny device.

**Statistical analysis of data**

Data obtained were expressed as mean ± Standard Error of Mean and were statistically analyzed using One-way ANOVA. The new Duncan Multiple Range test was used to compare the means of different groups. A *p*-value of $< 0.05$ was considered statistically significant.

## Results

### Comparative antibiotic susceptibility patterns of clinical and typed (ATCC 14028) isolates of *Salmonella typhi*

**Figure 1** compares the antibiotic susceptibility patterns of the clinical and typed (ATCC 14028) *S. typhi* isolates utilized in this investigation. The outcome demonstrated that all antibiotics utilized against the typed *Salmonella* sample had considerably larger inhibition zones than the clinical isolate ($p<0.05$). Ciprofloxacin exhibited the highest zone of inhibition (36.71±0.31 mm and 36.14±0.10 mm), followed by pefloxacin (33.18±0.21 mm and 28.77±0.06 mm). At the same time, tetracycline (18.72±1.42 mm) and chloramphenicol (11.61±0.73 mm) had the lowest zone of inhibition (against typed and clinical isolates, respectively).

### Comparative susceptibility patterns of clinical and typed (ATCC 14028) isolates of *Salmonella typhi* to *G. hirsutum* extract using agar well diffusion

**Figure 2** illustrates the differences in the sensitivity patterns to *G. hirsutum* extract between clinical and typed (ATCC 14028) isolates of *Salmonella typhi.* The diameter of the zone of inhibition of the extract at 800 mg/ml (1.42±0.33 mm) and ciprofloxacin (27.86±0.14 mm) against typed isolates was higher significantly ($p\leq0.05$) than clinical isolate at the same concentration (extract = 1.21±0.01 mm; ciprofloxacin= 21.07±0.06 mm). It was noted that the extract's inhibition zones were at concentrations of 400, 500, and 700 mg/ml.

### Comparative bactericidal effects of clinical and typed (ATCC 14028) isolates of *Salmonella typhi* to *G. hirsutum* extract using broth dilution

Comparative bactericidal effects of clinical and typed (ATCC 14028) isolates of *S. typhi* to *G. hirsutum* extract using broth dilution method are revealed in **figure (3)**. It was observed that the bactericidal activity of the extract is concentration dependent, and there were no significant ($p<0.05$) differences in the bactericidal activity of extract at 400 mg/ml (typed = 364.01±0.09 Cfu/ml; clinical= 378.63±0.31 Cfu/ml) and ciprofloxacin (typed = 18.31±0.11 Cfu/ml; clinical= 19.62±0.42 Cfu/ml) against both isolate, but the highest bactericidal effects wer observed in the extract at the 800 mg/ml (0.00±0.00 Cfu/ml) against both isolates.

### Minimum inhibitory concentration and Minimum bactericidal concentration of *G. hirsutum* extract

The MIC and MBC of *G. hirsutum* extract are reported in **table (1)**. The MIC of the extract against both isolates is 580 mg/ml while the MBC is 600 mg/ml.

### Acute toxicity effect of *G. hirsutum* extract on albino rats

*In-vivo* testing was done to determine the acute toxicity of *G. hirsutum* extract in Albino rats, and the results are displayed in **table (2)**. The acute toxicity effect was studied for seven days. At the end of the study, it was found that rats at concentrations of 10 and 100 mg/ml/kg body weight were very active and showed no signs of toxicity. In contrast, rats at concentrations of 1000 and 1500 mg/ml/kg body weight were hyperactive, and rats at concentrations of 2500 and 3500 mg/ml/kg body weight were anorexic. The acute toxicity assay lasted seven days without fatalities, and the acute toxicity concentration was determined to be 316.23 mg/ml/kg.

### Change in weight (g) of experimental animals during acute toxicity test

The change in weight (g) of experimental animals during the acute toxicity test is shown in **figure (4)**. It was observed that all the mice had significant ($p\leq0.05$) weight gain, with the highest weight gain observed at a concentration of 1500 mg/ml/kg (57.40±0.64 to 65.11±0.21) of body weight as illustrated in **figure (4)**.

### Change in body temperature (oC) of experimental animals during acute toxicity test

Change in body temperature ($^oC$) of experimental animals during acute toxicity test is presented in **figure (5)**. It was observed that at day seven, the extract contributed to a significant ($p\leq0.05$) increase in body temperature of experimental rats at a concentration of 100 mg/ml/kg of body weight.

### Effects of treatment with extract on fecal shedding of *S. typhi* (CFU/g)

**Figure 6** displays the effects of extract treatment on *S. typhi* fecal shedding (CFU/g). The results showed that the fecal shedding of the group infected and treated with 313.23 mg/ml/kg of extract (Group A) increased significantly ($p\leq0.05$) from 0.000.00 to 71.14±0.31× 103 Cfu/g before treatment and further significantly ($p\leq0.05$) decreased to 6.02±0.17× 103 Cfu/g after three days of treatment. In contrast, the group treated with 2 mg/ml/kg of ciprofloxacin had

a reduction of 1.62±0.91× 103 Cfu/g aftermaths of treatment.

**Effects of treatment with extract on organs invasion of *S. typhi* (CFU/g)**

**Figure 7** shows the effects of extract treatment *on S. typhi* organ invasion (Cfu/g). It was observed that the liver of the experimental rat in group A (infected and treated with extract 316.23mg/ml/kg) was heavily invaded by *S. typhi* after treatment compared to those in group C (treated with ciprofloxacin 2 mg/ml/body weight (kg) positive control) (2.03±0.33 Cfu/g), the kidney, heart, and intestine of the rat in group A was also invaded with 13.08. Additionally, compared to the rat in group D, the extract greatly decreased the invasion of *S. typhi* into important organs (infected but not treated).

**Body weight gain trend for Wistar rats treated with extract of *G. hirsutum***

**Figure 8** depicts the body weight gain trend for Wistar rats given *G. hirsutum* extract treatment. The results showed that group A (infected and treated with extract), group B (not infected but fed with extract), and group E (were neither infected nor treated) did not experience a significant change in the rat's body weight ($p \leq 0.05$), whereas group C (received ciprofloxacin) and group D (were infected but not treated) did experience a significant change in body weight ($p \leq 0.05$) at the conclusion of the treatment period.

**Effects of *G. hirsutum* extract on haematological parameters of Wistar rats infected with *S. typhi***

**Figure 9** shows the effects of *G. hirsutum* extract on blood parameters in Wistar rats infected with *S. typhi*. There was no discernible change in the PCV of the rats in groups A (infected and treated with extract), B (uninfected but fed extract), and E (not infected but fed extract) (were neither infected nor treated). Group C (those infected and got ciprofloxacin) had the greatest PCV levels, while group D had the lowest levels (were infected but not treated). Group A had the highest WBC (9.85±0.04×109/l), while group B had the lowest (2.55±0.11×109/l).

**Effects of *G. hirsutum* extract on differential white blood cell counts of Wistar rats infected with *S. typhi***

Effects of *G. hirsutum* extract on differential white blood cell counts of Wistar rats infected with S. typhi are shown in **figure (10)**. The result showed that the neutrophil and lymphocyte cell counts in groups A, B, C, D and E were 60, 61, 35, 48 and 60%; 33, 37, 62, 50 and 38% respectively.

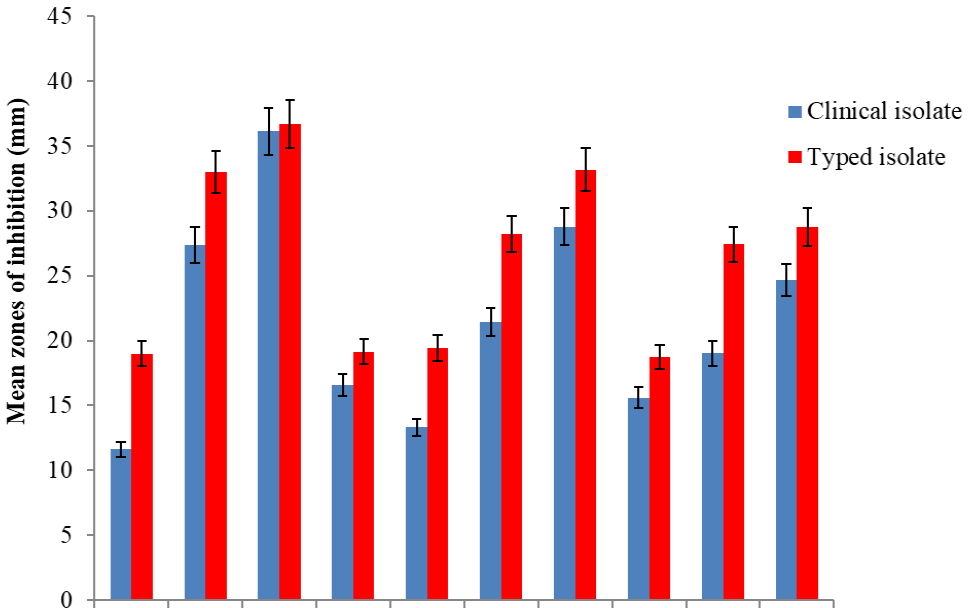
**Histological effects of *G. hirsutum* extract on Wistar rats infected with *S. typhi***

The histological effects of *G. hirsutum* extract on Wistar rats infected with ***S. typhi*** are shown in Plates 1 to 10 using liver and kidney histology. Plates 1 through 5 displayed the treatments' toxicological characteristics on the kidney of experimental rats. In comparison to rats in group E (Plate 5), which have normal kidney architecture, it was shown that the treatment had very modest pathological effects on the kidney of groups A to D (Plates 1 to 4) rats. Plates 6 to 10 display the treatment's effects on the livers of test rats. Rats in groups A to D (Plate 6 to 9) showed slightly different pathological effects from those in group E. (Plate 10), which has well-formed liver architecture.

**Fourier Transform Infrared Spectrophotometer (FTIR) spectra of *G. hirsutum* extract**

The FTIR spectra, spectral peak levels, and functional groups obtained for *G. hirsutum* leaf extract were displayed in **figure (11)** and **table (3)**, respectively. **Figure 11** shows the peaks produced at various wavelengths (cm-1). **Table 3** demonstrated that seven distinct peaks representing the functional group's alkene, anhydride, alcohol, sulfate, nitro compound, alkanes, and carboxylic acid were produced at wavelengths of 894.6, 1043.7, 1338.1, 1401.1, 1543.1, 2922.2, and 3268.9 cm-1, respectively.

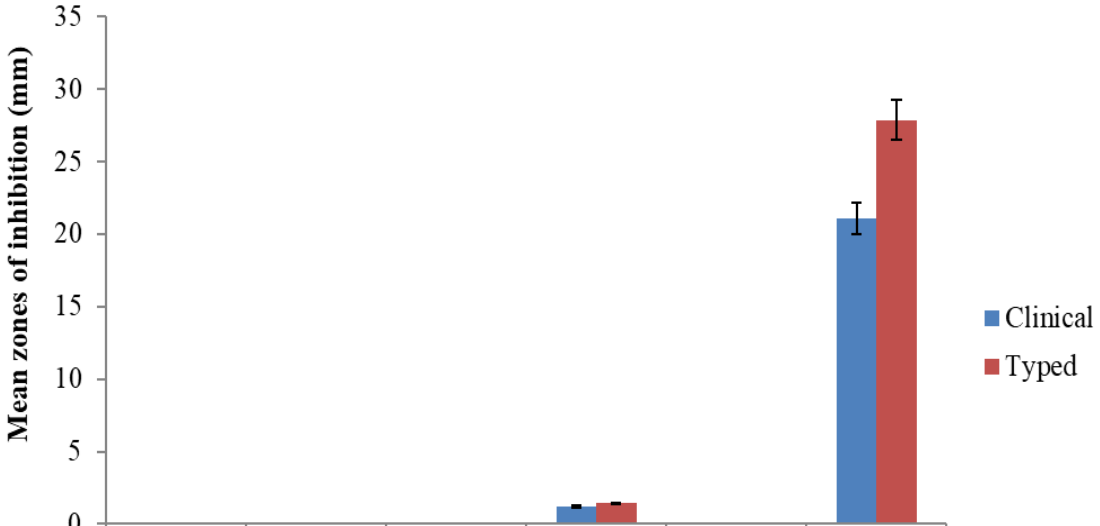
**Figure 1.** Comparative antibiotic susceptibility patterns of clinical and typed (ATCC 14028) isolates of *Salmonella typhi*




| | CHL | ZIN | CPX | AMX | AMP | GEN | PFX | TET | STR | SP |
|---|---|---|---|---|---|---|---|---|---|---|
| Clinical isolate | 11.61 | 27.36 | 36.14 | 16.59 | 13.32 | 21.42 | 28.77 | 15.61 | 19.02 | 24.64 |
| Typed isolate | 19 | 33 | 36.71 | 19.14 | 19.43 | 28.21 | 33.18 | 18.72 | 27.41 | 28.75 |

Keys: CHL= Chloramphenicol, ZIN= Zinnacef, CPX= Ciprofloxacin, AMX= Amoxicillin, AMP= Ampiclox, GEN= Gentamycin, PFX= Pefloxacin, TET= Tetracycline, STR= Streptomycin, SP= Septrin

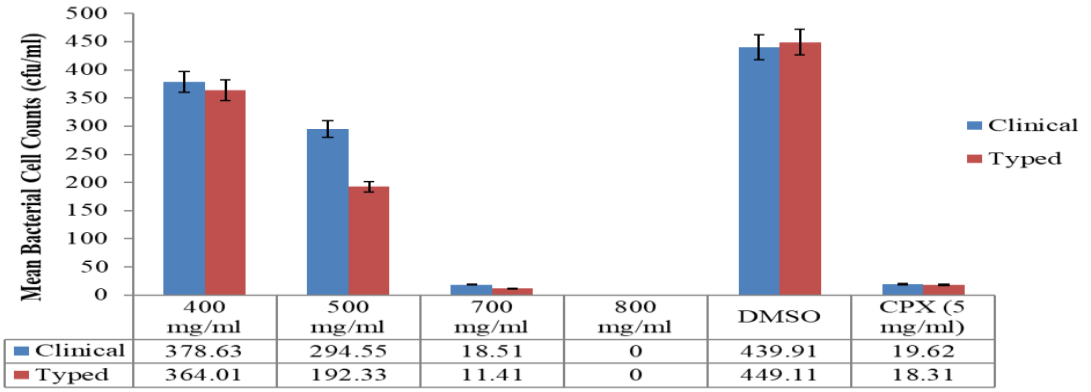
**Figure 2.** Comparative susceptibility patterns of clinical and typed (ATCC 14028) isolates of *Salmonella typhi* to *G. hirsutum* extract using agar well diffusion




| | 400 mg/ml | 500 mg/ml | 700 mg/ml | 800 mg/ml | DMSO | CPX (5 mg/ml) |
|---|---|---|---|---|---|---|
| Clinical | 0 | 0 | 0 | 1.21 | 0 | 21.07 |
| Typed | 0 | 0 | 0 | 1.42 | 0 | 27.86 |

Keys: CPX= Ciprofloxacin, DMSO= dimethylsulphoxide

**Figure 3.** Comparative bactericidal effects patterns of clinical and typed (ATCC 14028) isolates of *Salmonella typhi* to *G. hirsutum* extract using broth dilution




| | 400 mg/ml | 500 mg/ml | 700 mg/ml | 800 mg/ml | DMSO | CPX (5 mg/ml) |
|---|---|---|---|---|---|---|
| Clinical | 378.63 | 294.55 | 18.51 | 0 | 439.91 | 19.62 |
| Typed | 364.01 | 192.33 | 11.41 | 0 | 449.11 | 18.31 |

Keys: CPX= Ciprofloxacin, DMSO= dimethylsulphoxide

**Figure 4.** Change in weight (g) of experimental animals during acute toxicity test.

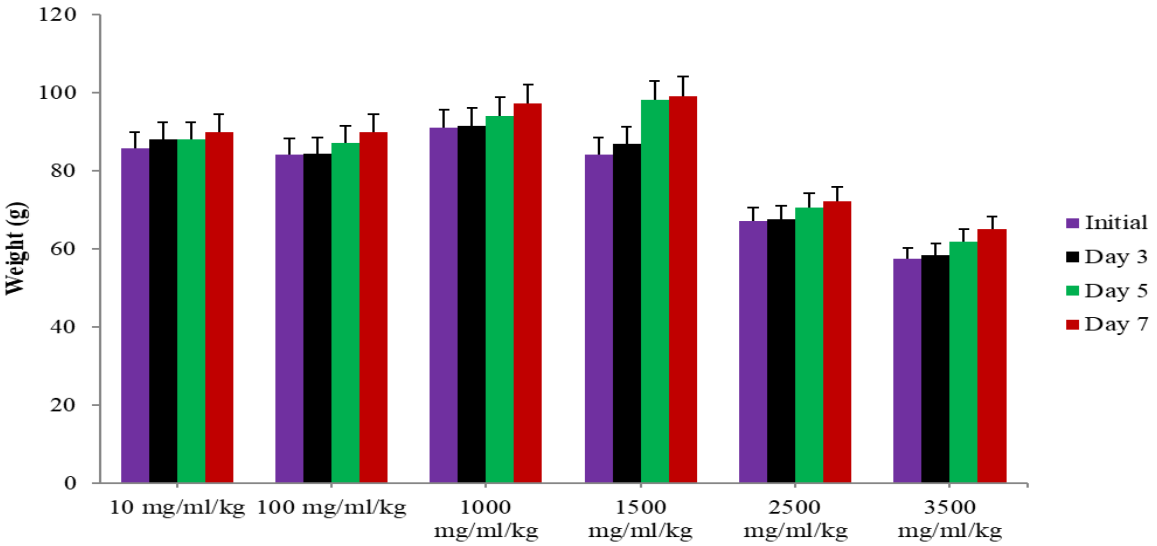



**Figure 5.** Change in body temperature (°C) of experimental animals during acute toxicity test.

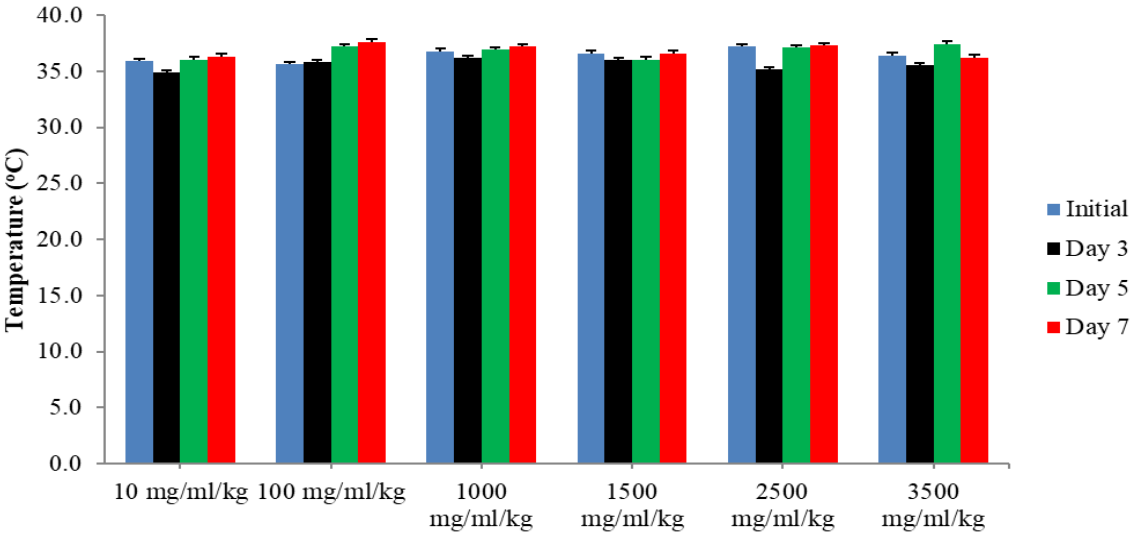
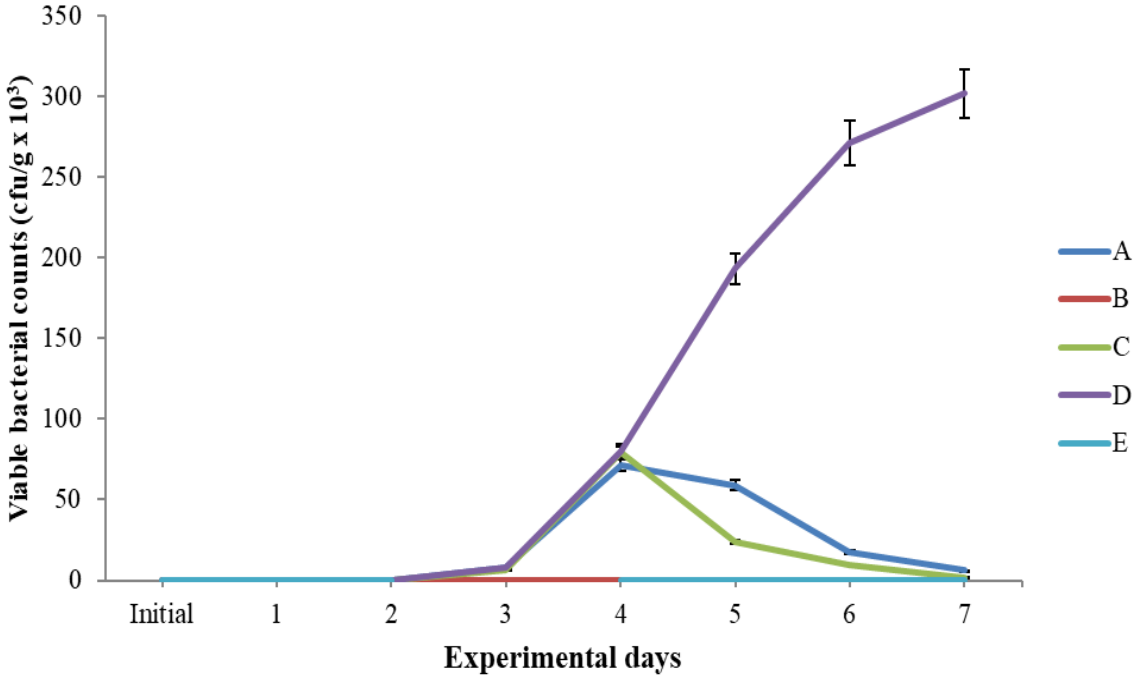
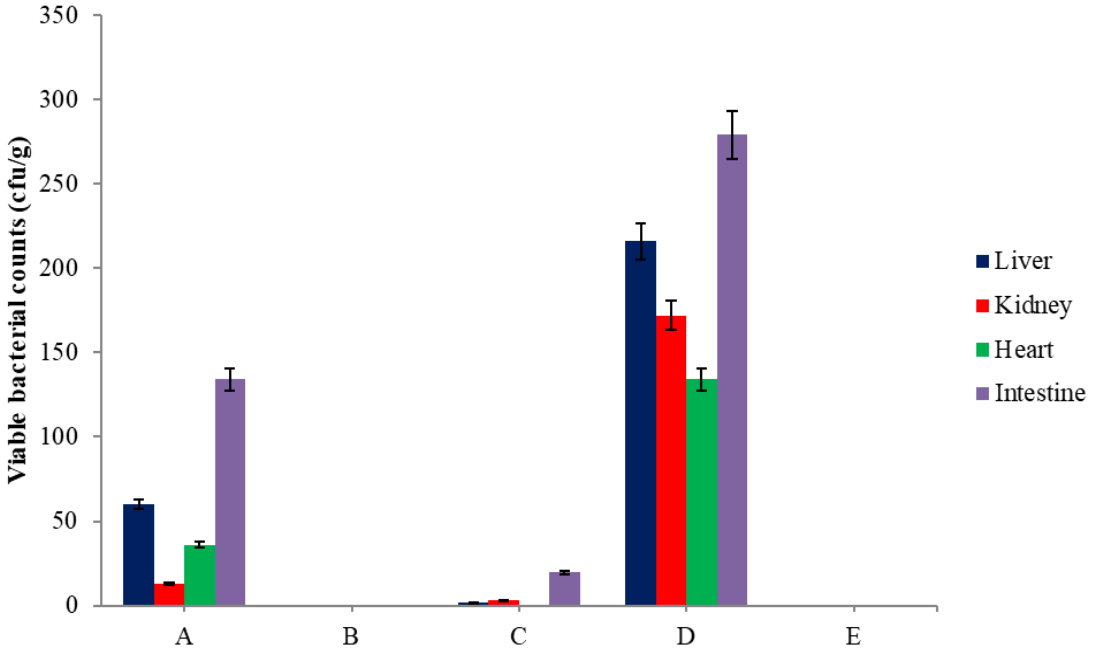


**Figure 6.** Effects of treatment with extract on fecal shedding of *S. typhi*(CFU/g)



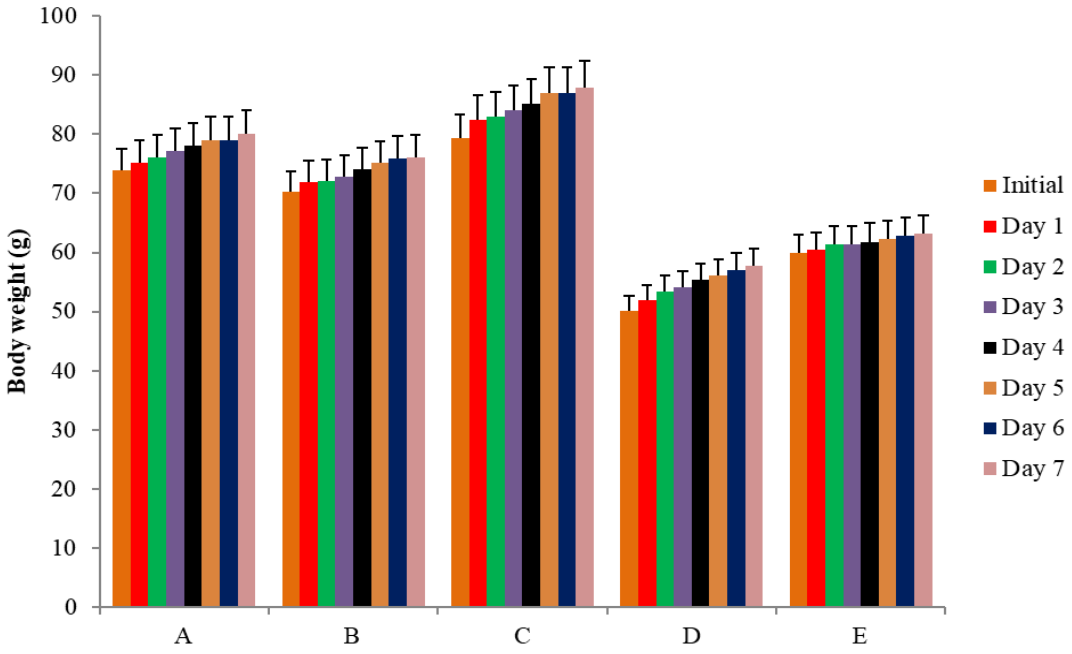

Key: Day 1 to 4 (infection), day 5 (start treatment)
Group A was infected and treated with the extract (316.23mg/ml/body weight (kg)
Group B was not infected but was fed with extract (316.23mg/ml/body weight (kg)
Group C received ciprofloxacin (2 mg/ml/body weight (kg) positive control groups.
Group D was infected but not treated (received distilled water during the treatment period) negative control group.
Group E was neither infected nor treated (they received distilled water during the treatment period)

**Figure 7.** Effects of treatment with extract on organs invasion of *S. typhi* (CFU/g**).**



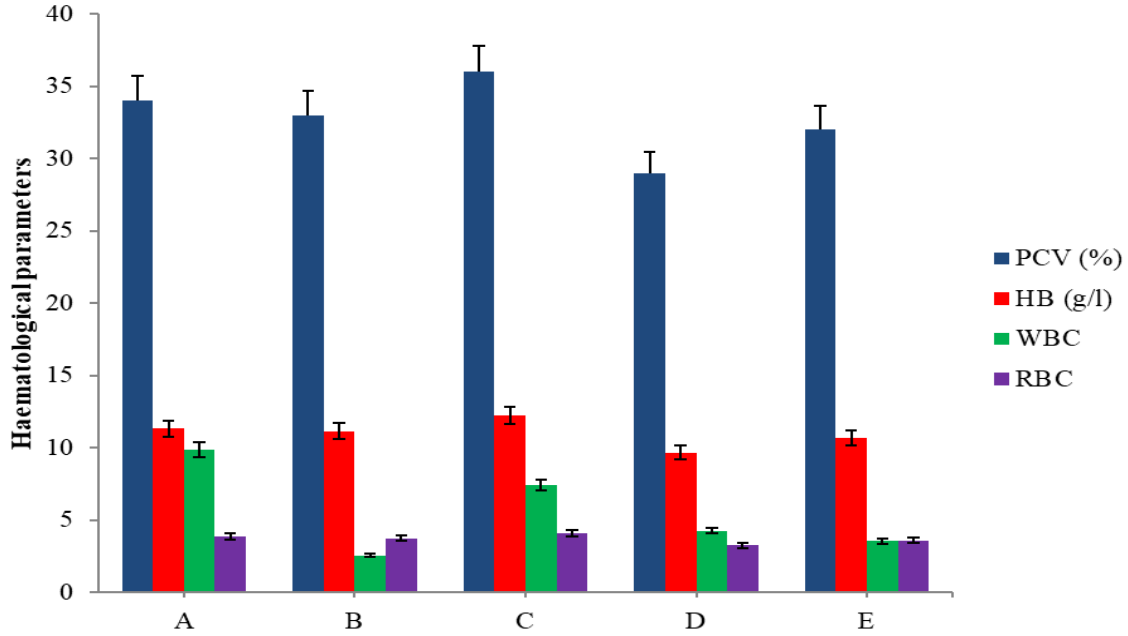

Key: Group A was infected and treated with the extract (316.23mg/ml/body weight (kg)
Group B was not infected but was fed with extract (316.23mg/ml/body weight (kg)
Group C received ciprofloxacin (2 mg/ml/body weight (kg) positive control groups.
Group D was infected but not treated (received distilled water during the treatment period) negative control group.
Group E was neither infected nor treated (they received distilled water during the treatment period)

**Figure 8 .** Body weight gain trend for Wistar rats treated with extract of *G. hirsutum.*



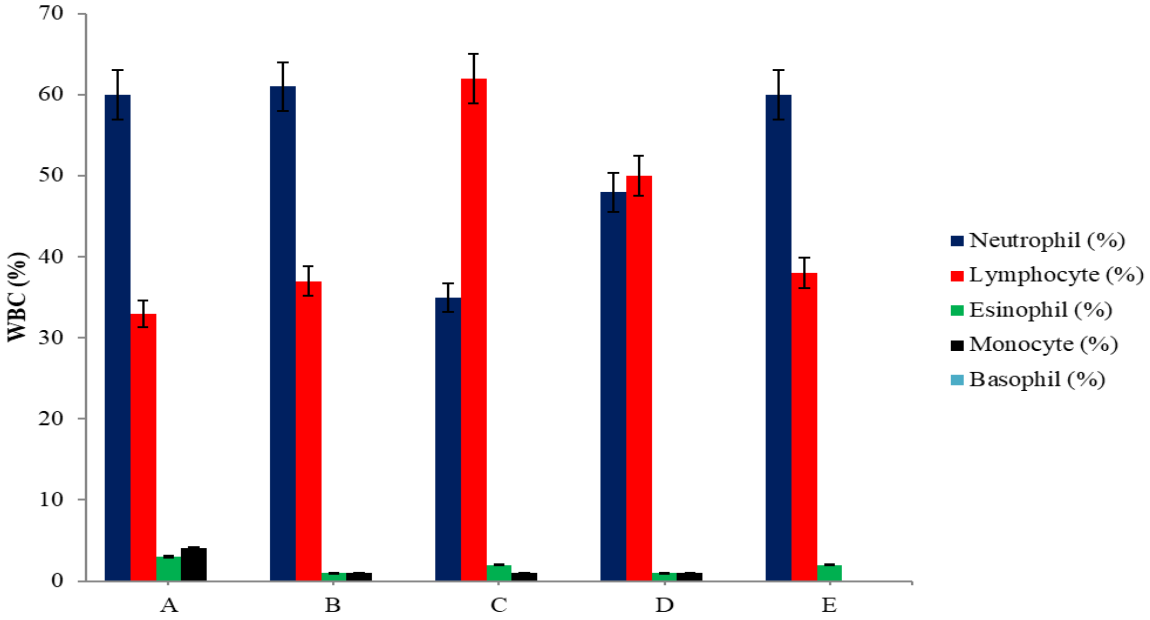

Key: Day 1 to 4 (infection), day 5 (start treatment)
Group A were infected and treated with extract (316.23mg/ml/body weight (kg)
Group B were not infected but fed with extract (316.23mg/ml/body weight (kg)
Group C received ciprofloxacin (2 mg/ml/body weight (kg) positive control groups.
Groups D were infected, but not treated (received distilled water during the treatment period) negative control group
Group E were neither infected nor treated (they received distilled water during the treatment period)

**Figure 9.** Effects of *G. hirsutum* extract on Haematological parameters of Wistar rats infected with *S. typhi*



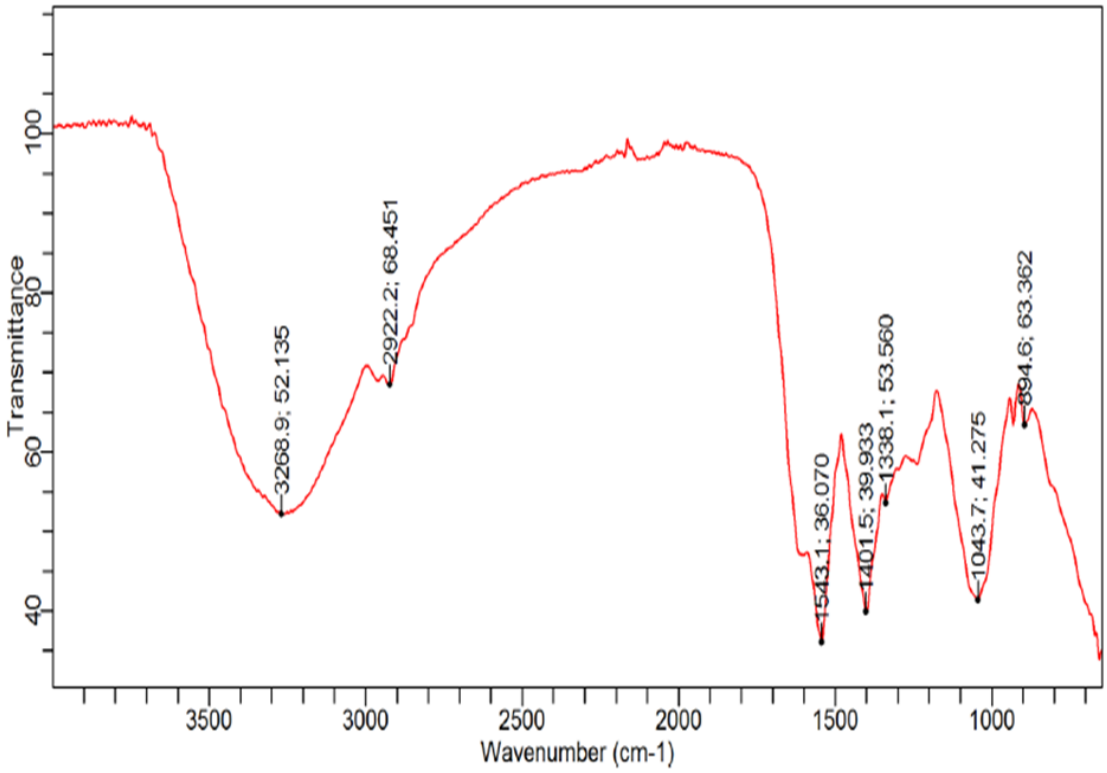

Key: PCV= packed cell volume, HB= haemoglobin, WBC= white blood cell ($10^9$/l), RBC= red blood cell ($10^{12}$g/l),
Group A was infected and treated with the extract (316.23mg/ml/body weight (kg)
Group B was not infected but was fed with the extract (316.23mg/ml/body weight (kg)
Group C received ciprofloxacin (2 mg/ml/body weight (kg) positive control groups.
Group D was infected but not treated (received distilled water during the treatment period) negative control group.
Group E was neither infected nor treated (they received distilled water during the treatment period)

**Figure 10.** Effects of G. *hirsutum* extract on differential white blood cell counts of Wistar rats infected with *S. typhi*.




Key: WBC = Differential White Blood Cell
Group A was infected and treated with the extract (316.23mg/ml/body weight (kg)
Group B was not infected but was fed with the extract (316.23mg/ml/body weight (kg)
Group C received ciprofloxacin (2 mg/ml/body weight (kg) positive control groups.
Group D was infected but not treated (received distilled water during the treatment period) negative control group

**Figure 11.** Fourier Transform Infrared Spectrophotometer (FTIR) spectra of *G. hirsutum* extract.



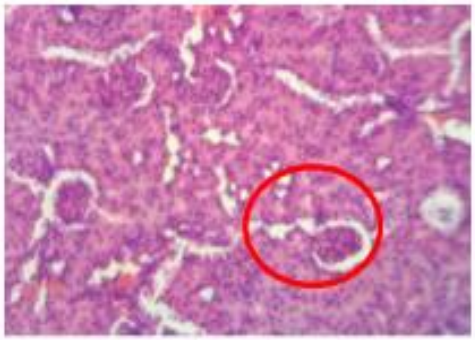


**Plate 1.** Kidney histopathology showing normal architecture of nephron and well-formed bowman capsule, possible deposition of immunological materials (IM) in the glomeruli basement.

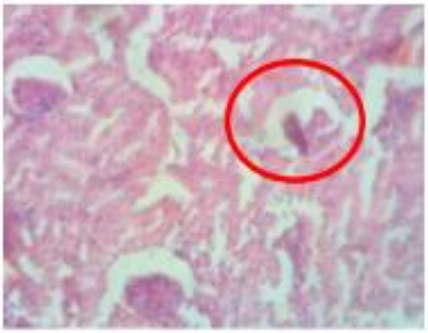


**Plate 2.** Kidney histopathology showing normal architecture of nephron and gradual loss of bowman capsule.

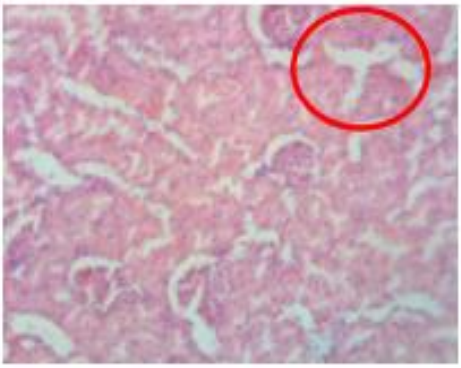


**Plate 3.** Kidney histopathology showing normal architecture of nephron and loss of bowman capsule.

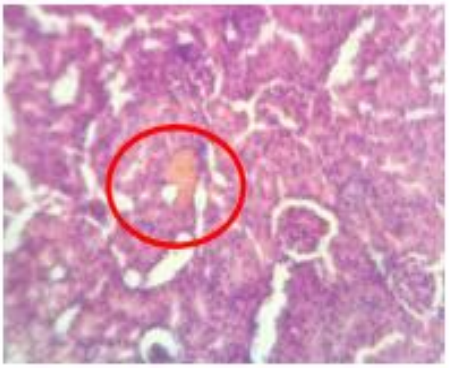


**Plate 4.** Kidney histopathology showing normal Architecture of nephron with intact glomeruli room (GR) and congregative acute tubular necrosis (TN) of the proximal convoluted tubule.

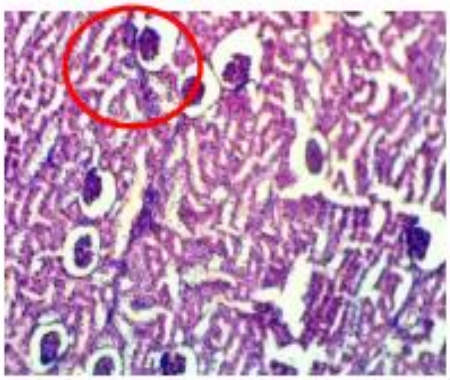


**Plate 5.** Kidney histopathology showing normal architecture of nephron and well-formed bowman capsule with intact glomeruli room.

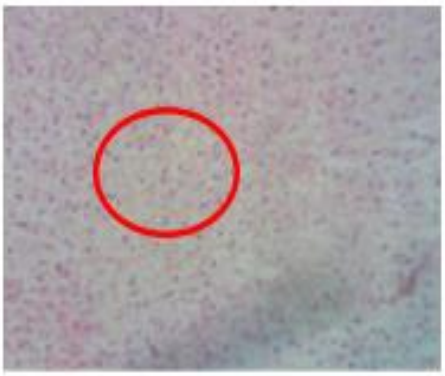


**Plate 6.** Liver with almost near normal architecture but with part of the sinusoid to be diffused and the hepatic sinusoids(S) separating the hepatic cord in place lined by Kupffer cells.

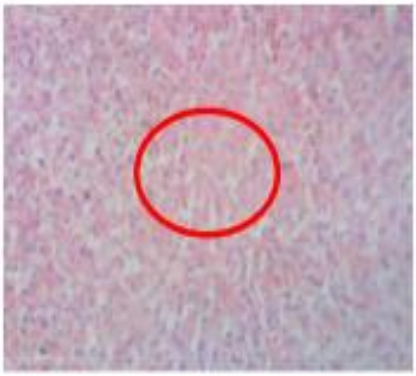


**Plate 7.** Liver with almost near normal architecture but with part of the sinusoid to be diffused.

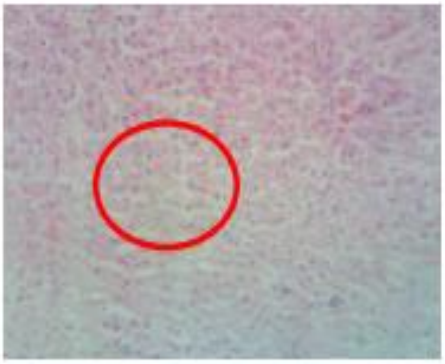


**Plate 8.** Liver with almost near normal architecture but with part of the sinusoid to be diffused.

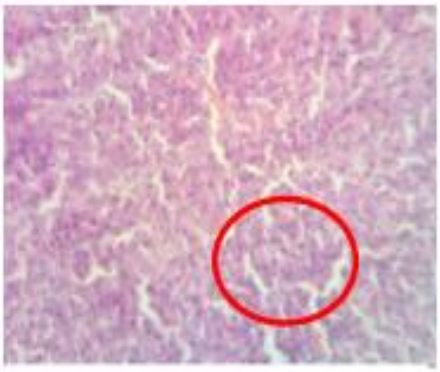


**Plate 9.** Liver with dilation of sinusoid

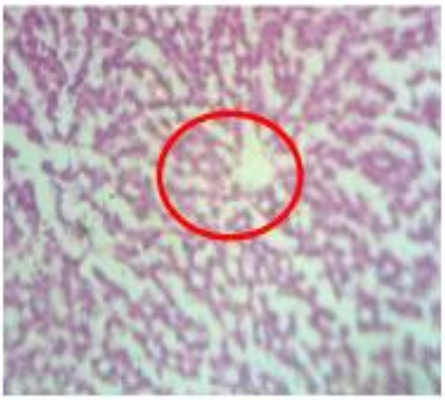


**Plate 10.** Normal liver structure with the hepatic cells in order and the hepatic sinusoids separating the hepatic cord in place lined by Kupffer cells.

**Table 1.** Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of *G. hirsutum* extract.

| ***Salmonella typhi* Isolates** | **MIC (mg/ml)** | **MBC (mg/ml)** |
|---|---|---|
| Clinical | 580 | 600 |
| Typed | 580 | 600 |

Key: MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration

**Table 2.** Acute toxicity effect of *G. hirsutum* extract on Albino rats.

| **Dosage/body weight (kg)** | **Day 1** | **Day 3** | **Day 5** | **Day 7** |
|---|---|---|---|---|
| 10 mg/ml/kg | Very active and no sign of toxicity | Very active and no sign of toxicity | Very active and no sign of toxicity | Very active and no sign of toxicity |
| 100 mg/ml/kg | Very active and no sign of toxicity | Very active and no sign of toxicity | Very active and no sign of toxicity | Very active and no sign of toxicity |
| 1000 mg/ml/kg | Very active and no sign of toxicity | Very active and no sign of toxicity | Very active and no sign of toxicity | Very hyperactive |
| 1500 mg/ml/kg | Very active and no sign of toxicity | Very active and no sign of toxicity | Very hyperactive | Very hyperactive |
| 2500 mg/ml/kg | Very active and no sign of toxicity | Very active and no sign of toxicity | Very hyperactive | Anorexia was observed |
| 3500 mg/ml/kg | Very active and no sign of toxicity | Very active and no sign of toxicity | Anorexia was observed | Anorexia was observed |

Acute toxicity was calculated to be 316.23mg/ml/kg

**Table 3.** FTIR spectral peak values and functional groups obtained for leaf extract of *G. hirsutum.*

| **S/N** | **Peak values ($cm^{-1}$)** | **Functional group** | **Interpretation** |
|---|---|---|---|
| 1 | 894.6 | C=C bending | Alkene |
| 2 | 1043.7 | CO-O-CO stretching | Anhydride |
| 3 | 1338.1 | O-H bending | Alcohol |
| 4 | 1401.1 | S=O stretching | Sulphate |
| 5 | 1543.1 | N-O stretching | Nitro compound |
| 6 | 2922.2 | C-H stretching | Alkanes |
| 7 | 3268.9 | O-H stretching | Carboxylic acid |

Key: C= Carbon, O= Oxygen, S= Sulphur, N= Nitrogen, H= Hydroge

**Discussion**

This study examined the antibacterial activity against *S.typhi*, and the extracts of *G. hirsutum* leaves extracted with carbonated beverage "7UP". Ciprofloxacin had the highest zone of inhibition, followed by pefloxacin against typed and clinical isolates. In contrast, the least zone of inhibition was observed in tetracycline and chloramphenicol against typed and clinical isolates, respectively. Generally, zones of inhibition against clinical isolates were smaller than that of typed isolates. This could be because the clinical isolate has developed resistance against the antibiotics used [30]. The susceptibility result of test bacteria to standard antibiotics revealed that ciprofloxacin had a higher inhibitory effect than the extract using agar well diffusion. The higher antibacterial activity of model antibiotics is not surprising since the antibiotics are in a refined state. The standard antibiotics used in this study are the first-line drugs employed in treating typhoid fever [31]. However, using the broth dilution method, extract at a higher concentration of 800 mg/ml had better inhibitory potential than the ciprofloxacin.

The bioactive components in the extract may not have diffused into the agar in the agar well but instead were able to directly kill microbial cells in broth, explaining the variations in the anti-Salmonella efficiency of the extract in the agar well and tube dilution method. The active components of the extract were found to kill microbial cells in broth dilution, but they did not diffuse into MHA, as has been previously reported by other investigations [32,33]. Further evidence that the extracts are bacteriostatic at low concentrations and bactericidal at high concentrations comes from the MBC values obtained from the extracts of both plants against the test bacteria, which were greater than the MIC values. **Nwanchukwu et al.** [34] claimed that the level at which crude extracts inhibited test organisms is used to investigate the efficacy of chemotherapeutic agents under standard conditions.

The findings of this study's antibacterial screening support the historic usage of these plants as medicines for treating gastroenteritis and other bacterial illnesses [35,36]. The acute toxicity assay lasted seven days without fatalities, and the acute toxicity concentration was 316.23 mg/ml/kg. However, at greater concentrations, anorexia and hyperactivity in the rats were seen. Rats' anorexia and hyperactivity indicated that the extract had a central stimulant effect, consistent with earlier findings [37]. After three days of therapy, fecal shedding in the infected group (Group A) that received 313.23mg/ml/kg of extract decreased compared to the untreated group. Since an increase was seen in the negative controls, the extract's combined effects may cause a considerable reduction in bacterial load in infected animals after the start of the treatment (infected and untreated). This supports **Pandey et al.** [38] findings that the extract decreased the bacterial load in vivo. The kidney, heart, and gut of the treated group were invaded. This invasion may have occurred because the extract could not remove *Salmonella* from the body of the experimental animal.

After the treatment period, there was no significant ($p \leq 0.05$) change in the rat's body weight in groups A (infected and treated with extract), B (not infected but fed with extract), or E (were neither infected nor treated), but there was a significant ($p \leq 0.05$) increase in body weight in groups C (received ciprofloxacin), and D (were infected but not treated). Rats in the untreated but infected group may have gained weight due to water buildup in the intestines and indigestion from meals [39]. Effects of G. hirsutum extract on *S. typhi*-infected Wistar rats' hematological parameters. The hematological status after 3 days of oral extract administration revealed that there was generally no significant difference in the PCV of rats in groups A (were infected and treated with extract), B (were not infected but fed with extract), and E (were neither infected nor treated), demonstrating that the extract did not contribute to the increase in red blood cells of the experimental animal, with the least PCV observed in the group infected and not treated could possibly be attributed to the various pathophysiological effects produced during *Salmonella* infection.

*Salmonella* could multiply in rats under the control of the Nramp gene, which could result in chronic cell lysis because of the presence of free radicals generated during inflammatory responses **Brown et al.** [40], **Donald et al.** [39], **Droy-Lefaix and Bueno,** [41]. It has been demonstrated that an increase in parasitemia always corresponds strongly with the degree of anemia [42]. Compared to rats in group E (not infected or treated), with normal kidney architecture, it was shown that the treatment of the kidney of group A through D rats had modest pathological effects on the liver and kidney. However, the presence of degenerative consequences in the group fed with extract alone and

the possibility of Salmonella entering the organ could also account for toxicity. This study is not in support of the findings of **Soniran et al.** [43], which stated that the administration of antimalarial drugs reduces the extent of damage caused by parasites on the organs..

## Conclusion

Study findings show that G. hirsutum extract has anti-*Salmonella* activity, possibly due to the individual or combined effects of the phytoconstituents it contains. The extract showed stronger anti-Salmonella activity and was found to compete favorably with the commercial antibiotic ciprofloxacin in-vitro. The overall findings of this study offer fundamental knowledge for the potential use of *G. hirsutum* leaf extract, extracted with carbonated beverage "7UP," in the treatment of salmonellosis, particularly typhoid fever. According to acute toxicity and histopathology, the extract may be harmful in addition to antibacterial action. The anti-Salmonella action of the extract was demonstrated by the FTIR spectra, which included functional groups such as alkene, anhydride, alcohol, sulfate, nitro compound, alkanes, and carboxylic acid. The use of '7UP' as an extraction solvent for medicinal plants in our community for treating diverse medical ailments, especially infectious diseases, is justified. However, it could pose a toxicological effect.

## Competing interests

The authors declare that they have no competing interests.

## Funding

No funding was obtained for this study.

## Authors' contribution

'FOO designed and supervised the study. ''TGE developed the methodology and
Literature, conducted the study, acquired, analyzed and interpreted the data obtained. TGE
Wrote the first draft. ''TGE, AIO and MOE previewed and fine-tuned the draft before
Submission''. All authors have read and approved the manuscript.

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