# *In-vitro* Anti-*Salmonella* Activity of *Gossypium hirsutum* Leaves Extracted with Lime Juice


## ABSTRACT

Invasive *Salmonella* infections are responsible for a significant burden of morbidity and mortality worldwide. There has been increase in resistance to anti-typhoid prescription drugs medicinal plants have gained popularity among both urban and rural dwellers in the treatment of not only typhoid fevers but also to treat various ailments. The present study was undertaken to investigate the anti-*Salmonella* activity of *Gossypium hirsutum* leaf extract on *Salmonella typhi* (clinical isolates) using fresh lime juice as an extraction solvent. Extraction of lime juice, bioactive components of the plant leaf and *in vitro* anti-*Salmonella* activity of extract were all carried out using standard microbiological methods. *Salmonella typhi* ATCC 14028 (Type isolates) was used as control. Fourier Transform Infrared Spectrophotometer (FTIR) was used to identify the functional groups in the extract. The result revealed that clinical (mean diameter of inhibition zone 35.10±0.45 mm) and typed (mean diameter of inhibition zone 36.71±0.32 mm) isolates showed highest susceptibility to ciprofloxacin. The crude extract showed an inhibition zone ranging from 24.63±0.11 to 1.43±0.04 mm for clinical and 25.11±0.62 to 1.18±0.31 mm for typed isolates at 600 and 200 mg/mL, respectively. Fourier Transform Infrared Spectrophotometer (FTIR) indicated the presence of various functional groups in the extract such as 1,2,4-trisubstituted arenes, sulfoxide, vinyl ether, phenol, carboxylic acid, conjugated alkene, α, β-unsaturated ester, allene, isothiocyanate and alcohol. The overall results indicated that the extract obtained from *G. hirsutum* leaves using a lime juice has the potential for an effective treatment of salmonellosis, including typhoid fevers. However, it is necessary to ascertain the safety of this extract *in vivo* in further studies.



Key words: *Salmonella*, lime juice, leaf, crude extract, functional group


## Introduction

*Salmonella* spp. is the causative agent of salmonellosis. It is a rod-shaped gram-negative facultative anaerobe bacterium belonging to the *Enterobacteriaceae* family. Among 2,300 closely-related *Salmonella* serovars recognized, *Salmonella typhi* and *paratyphi* are solely human pathogen, they cause systemic infections and typhoid fever, whereas others species such as *S. Typhimurium* cause gastroenteritis (Zhang *et al.*, 2008; Kirk *et al.*, 2015).

Salmonellosis is more prevalent in some developing areas of continents such as Africa, Asia, and South America.

In humans, salmonellosis is seen in two kinds viz. enteric fever which can be typhoid or paratyphoid and gastroenteritis which is non-typhoidal. Typhoid fever is an acute, life-threatening febrile illness caused by the bacterium *S. typhi* and *paratyphi*. There are estimated 20 million cases and 200,000 deaths worldwide have been reported each year (Ao *et al.*, 2015).

Typhoid fever causes a serious health problem, although it could be treated with antibiotics, however, due to increase in resistance of *S. typhi* to conventional antibiotic therapy, there is a need to search for alternative therapeutic methods of treatment (Kirk *et al.*, 2015).

Therefore, the search for new or alternative therapeutic methods becomes imperative in treating infection caused by this organism.

In folklore medicine, especially in some communities in Southwest of Nigeria, typhoid fever is treated locally with a traditional decoction made from the leaves of *Psidium guavaja*, *Azadirachta indica*, *Gossypium hirsutum*, *Mangifera indica* and *Persea americana*, the bark of *Anacardium occidentale* and *Swietenia mahagoni* and the husk of *Cocos nucifera* (Kraft, 2009; Modi *et al.*, 2007; Moquin *et al.*, 2009).

In the previous study on anti*Salmonella* activity of *Euphorbia heterophylla* aqueous extract, cassava flakes (white and yellow) and the combinations of *Euphorbia heterophylla* + white cassava flakes. It was reported that combinations of *Euphorbia heterophylla* + white cassava flakes had the highest anti*Salmonella* activity *in-vitro* while the result of the *in-vivo* studies showed that *Euphorbia heterophylla* mixed with cassava flakes can be used in the control of *Salmonella typhi* infections, and reversed the histopathological damages caused by *Salmonella typhi* in the liver and kidney of experimental rats. The study concluded that *Euphorbia heterophylla* aqueous extract and cassava flakes can be used to treat disruptions in the kidney and liver with mild histopathological features on liver, heart and kidney of mice (Omoya *et al.*, 2015).

Many organic solvents, hot and cold water have been used as extraction solvent to assay antibacterial efficacy of different medicinal plants (Egharevba and Ikhatua, 2008). However, it is commonly practice among the 'Yoruba' tribe to extract bioactive components of leaf, root and stem of ethno-medicinal plants with lime juice or taken the decoction (how the decoction is obtained) in combinations with lime juice (Ene *et al.*, 2010). The present study was undertaken to investigate anti-*Salmonella* activity of *Gossypium hirsutum* leaf extract on *Salmonella* Typhi using fresh lime juice as extraction solvent with the view to provide scientific evidence for its application as a medicinal plant. During this study, it was observed that there has not been any previous published literature on extraction of plant with lime juice as extraction solvent and examination of phytochemicals using FTIR, therefore this study is a baseline study for further studies.

## Materials and method

### Collection of leaves of *Gossypium hirsutum*

Fresh leaves of *Gossypium hirsutum* were collected before the sunrise to prevent plant photo-oxidation around the North gate of Federal University of Technology, Akure. The leaves that have no injury or chlorosis were sorted out and kept in a clean sack for further work. The plant leave was identified by an expert, plant scientist in the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure Ondo State. The vernacular name of this plant is "ewe owu" by Yoruba's' in the South western part of Nigeria.

### Selection and Extraction of Lime Juice

Lime that was free of decay and mold was taken. It was washed with distilled water several times to remove soil and other extraneous matter and then surfaced sterilized with 70% ethanol. The fruits were then halved (using a presterilized knife) and the juice squeezed with presterilised juice extractor aseptically (sterile gloves worn during operation) into sterile 100

mL conical flasks. In order to determine that the lime juice is not contaminated with microorganism, the lime juice was filtered with Millipore membrane filter facilitated with vacuum pump after which a loop-full of the lime juice was inoculated on nutrient agar and potato dextrose agar plates to confirm the sterility. The presence of zero microbial loads indicates that the lime is sterile. The fresh lime juice was kept at -4 $^{o}$C for further use.

**Preparation of plant extract**

Plant extract was prepared as per the method reported in the literature (Tomassini *et al.* 2009). The fresh leaves were washed with sterile distilled water and air dried until they turned brittle and fully crispy. The dry leaves were crushed manually using clean mortar and pestle, then pulverized into fine powder by a blending machine (Philips HR2001). They were separately kept in an airtight container to avoid the absorption of moisture. The powdered samples were soaked for 72 hours in fresh lime juice in ratio of 1:10 each (i.e. 50 g of the powdered sample in 500 mL of lime juice) as solvents used for the extraction of the bioactive compounds from the plants. After 72 hours, it was sieved using muslin cloth and then filtered using Millipore filter paper. The filtrates were vaporized to dryness using rotary evaporator (Union Laboratories England). The extracts were preserved in a sterile bottle at -4 °C ready for use (Ogoti *et al.*, 2015).

**Test organism**

The clinical bacterial strains (*Salmonella typhi*) were obtained from the culture collection bank, Department of Microbiology, Federal University of Technology Akure and the source of the clinical isolate was human stool. Clinical isolate of *Salmonella typhi* and typed (ATCC 14028) *Salmonella typhi* was used as control. The isolates were confirmed based on cultural, morphological and biochemical characteristics following standard methods of identifying *Salmonella typhi* (Cheesbrough, 2014). The bacterial strain was grown in nutrient broth for

12-18 hours at 37°C on rotary shaker. Cells were grown at 37°C for 18 hours and the cultures were kept at 4°C.

### *In vitro* Antimicrobial susceptibility tests

#### Standardization of the inoculum

The inoculum was prepared by inoculating colonies of fresh test cultures into sterile distilled water. The turbidity was compared to 0.5McFarland standard, which was prepared according to method reported in literature Cheesbrough (2014).

#### Antibiotics susceptibility test using commercial antibiotics

Antibiotics sensitivity test of the bacterial isolates were determined by disc diffusion method as described by Cheesbrough (2014). Standard inoculum of 18 hours broth was spread on Muller Hinton agar using sterile swab in triplicate. The antibiotic discs were placed on the plate at equidistance. The plates were then incubated for 18 hours at 37$^{o}$C and diameter of zone of inhibition were measured and recorded in millimeter (mm). The following commercial antibiotics discs (Fondoz Laboratories Ltd, Nigeria) were used; Chloramphenicol (CH) 30 µg, Zinacef (SP) 20 µg, Ciprofloxacin (CPX) 10µg, Amoxicillin (AM) 25µg, Ampiclox (AMP) 30µg, Gentamycin (GEN) 10µg, Pefloxacin (PEF) 5µg, Tetracycline (TET) 5µg, Streptomycin (S) 10 µg and Septrin (SXT) 30µg.

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

#### Agar well diffusion test

The extracts were dissolved and diluted using 30 % volume/volume (v/v) dimethylsulphoxide (DMSO) to obtain different concentrations 200, 300, 400 and 600 mg/mL. Surface of solidify Muller Hinton agar was aseptically streaked with the standerdised (0.5McFarland turbidity standard) inoculum of test organism. The 200 mg/mL, 300 mg/mL, 400 mg/mL and 600 mg/mL of the extracts of *G. hirsutum* (100 µL) leaves were introduced into the wells earlier bored with sterile cork borer on Muller Hinton agar

plate. The plates were incubated aerobically at 37$^{o}$C and examined after 24 hours. The plates were examined for microbial growth inhibition and the Inhibition Zone Diameter (IZD) was measured to the nearest millimeter and compared with those produced by the commercial antibiotic ciprofloxacin which was used as control. All experimental procedures were performed in triplicates.

**Broth dilution test**

The effects of extract on anti-*Salmonella* efficacy in broth was assayed, 10.0 mL of Muller Hinton broth was prepared in a test tube and inoculated with 10 µl of *Salmonella* (0.5McFarland turbidity standard) was inoculated into the broth and treated with 100 µl of varying concentrations of extract inside the test tube and incubated at 37 $^{o}$C for 18 hours after which the test tube was shaken and 100 µl was pour plated on nutrient agar, incubated at 37 $^{o}$C for 24 hours and number of colony was counted after incubation period (Marcelin *et al.*, 2016).

**Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) of *G. hirsutum* Extracts**

The Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) of the extracts were determined using the broth (tube) dilution technique (Anibijuwon and Udeze, 2009). Dilutions of the extract in Mueller Hinton broth were prepared in tubes. The concentration of inoculum was also standardized to 0.5 McFarland's turbidity. The Mueller Hinton broth in tubes containing the different concentration of plant extract, 200 mg/mL, 300 mg/mL, 400 mg/mL and 600 mg/mL were then inoculated with 0.5 ml of the standardized culture. The tubes were then incubated at 37°C for 24 hours. MIC and MBC values were recorded.

**Identification of functional groups of the plant extract by FTIR**

Dried powder of extract was used, 10 mg of the extract was encapsulated in 100 mg of KBr pellet in order to prepare translucent sample discs. The prepared sample was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan) with a scan range from 400 to 4000 $cm^{-1}$ (Ashokkumar and Ramaswamy, 2014). Different peaks generated were interpreted by the expert in the Department of Chemistry, Federal University of Technology, Akure.

**Statistical analysis of data**

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

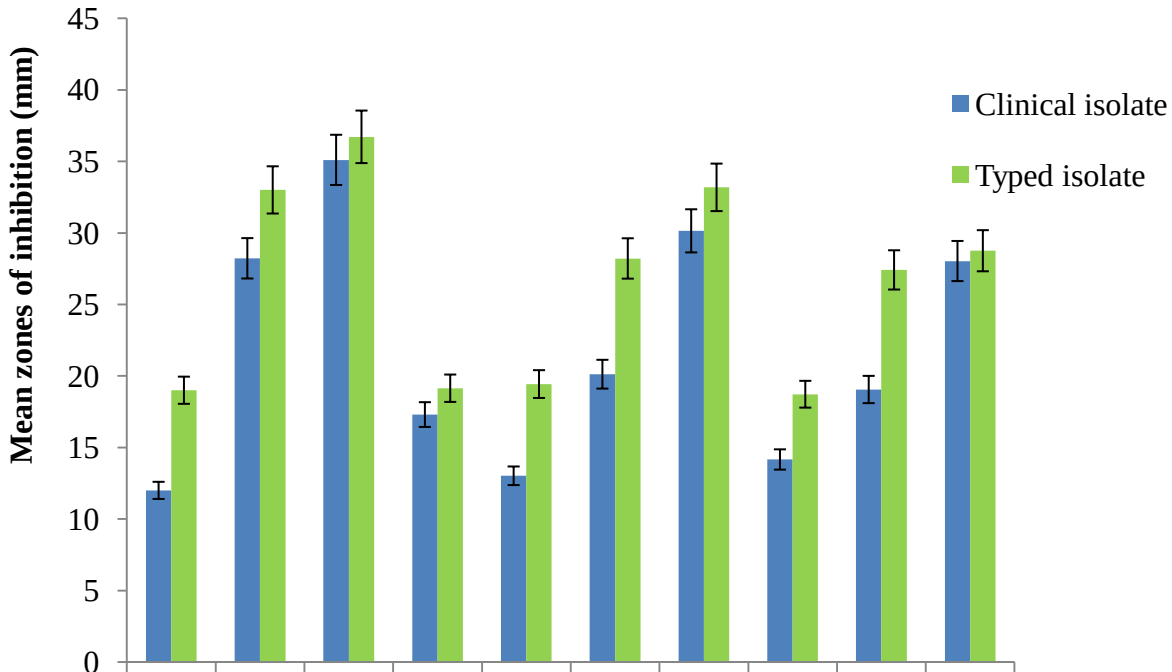
**Results**

**Comparative Antibiotic Susceptibility Patterns of Clinical and Typed (ATCC 14028) Isolates of *Salmonella* Typhi using disc diffusion method**

Figure 1 revealed the comparative antibiotic susceptibility patterns of clinical and typed (ATCC 14028) isolates of *Salmonella*Typhi used for this study. It was noted that *Salmonella* Typhi clinical and typed isolates exhibited varying susceptibility to antibiotics, ciprofloxacin inhibited both clinical (35.10±0.45 mm) and typed (36.71±0.32 mm) isolates than other antibiotics used and there was no significant ($p<0.05$) difference between the zones of inhibition showed by ciprofloxacin while chloramphenicol (12.00±0.01 mm) had least inhibition against clinical isolate and tetracycline (18.73±0.32 mm) had the least inhibition against typed isolate.

**Comparative Susceptibility Patterns of Clinical and Typed (ATCC 14028) Isolates of *Salmonella* Typhi to *G. hirsutum* Extract Using Agar Well Diffusion**

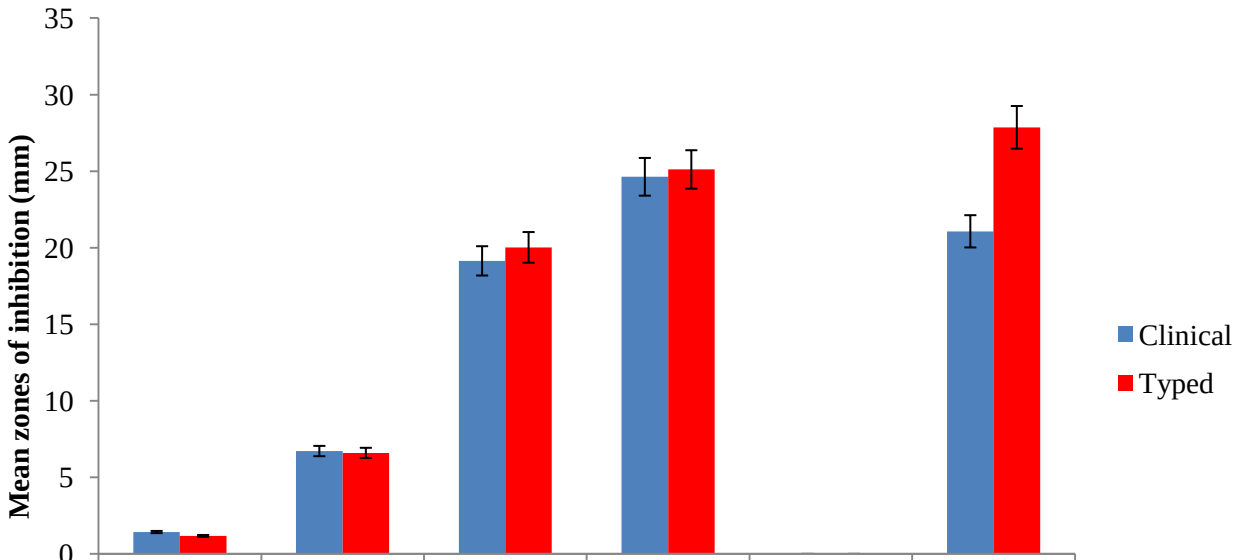
Comparative susceptibility patterns of clinical and typed (ATCC 14028) isolates of *Salmonella* Typhi to *G. hirsutum* extract using agar well diffusion method is shown in Figure 2. The result showed that the anti-*Salmonella* efficacy of the extract is concentration dependent, the extract had no significant ($p<0.05$) different in the inhibition of clinical and typed isolates at concentration of 300, 400 and 600 mg/mL. however, the highest inhibition against clinical isolate was observed at extract concentration of 600 mg/mL (24.63±0.11 mm) and the least at 200 mg/ml (1.43±0.04 mm) while the highest and the least extract concentration that inhibited typed isolate was 600 (25.11±0.62 mm) and 200 (1.18±0.31 mm) mg/ml respectively. Ciprofloxacin was used as control and the zones of inhibition against clinical and typed isolates were 21.07±0.06 and 27.86±0.03 mm.




| | CHL | ZIN | CPX | AMX | AMP | GEN | PFX | TET | STR | SP |
|---|---|---|---|---|---|---|---|---|---|---|
| Clinical isolate | 12 | 28.22 | 35.1 | 17.3 | 13.02 | 20.12 | 30.14 | 14.16 | 19.05 | 28.03 |
| Typed isolate | 19 | 33 | 36.71 | 19.14 | 19.43 | 28.21 | 33.18 | 18.72 | 27.41 | 28.75 |

**Figure 1: Comparative Antibiotic Susceptibility Patterns of Clinical and Typed (ATCC 14028) Isolates of *Salmonella* Typhi (disc diffusion assay)**

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




| | 200 mg/ml | 300 mg/ml | 400 mg/ml | 600 mg/ml | DMSO | CPX (5 mg/ml) |
|---|---|---|---|---|---|---|
| Clinical | 1.43 | 6.72 | 19.14 | 24.63 | 0 | 21.07 |
| Typed | 1.18 | 6.6 | 20.02 | 25.11 | 0 | 27.86 |

**Figure 2: Comparative Susceptibility Patterns of Clinical and Typed (ATCC 14028) Isolates of *Salmonella*Typhi to *G. hirsutum* Extract Using Agar Well Diffusion**

CPX= Ciprofloxacin, DMSO= dimethylsulphoxide

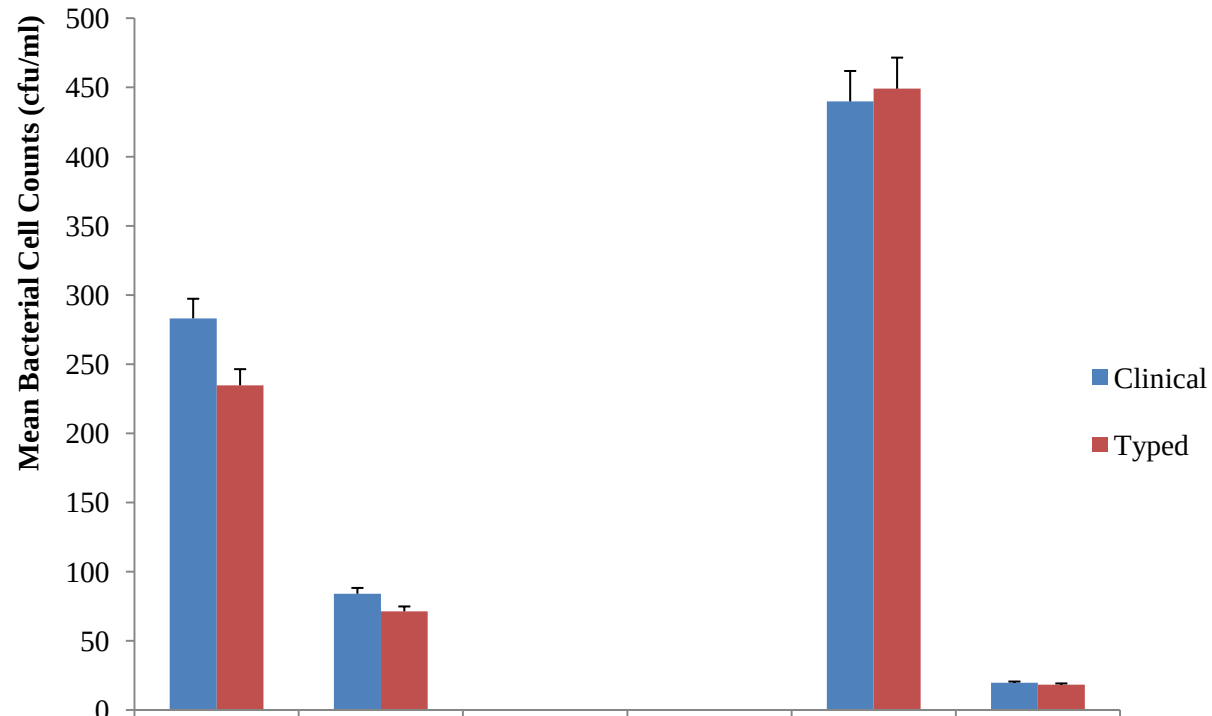
**Comparative Bactericidal Effects of *G. hirsutum* Extract on Clinical and Typed (ATCC 14028) Isolates of *Salmonella*Typhi Using Broth Dilution**

Comparative bactericidal effects of *G. hirsutum* extract on clinical and Typed (ATCC 14028) isolates of *Salmonella*Typhi using broth dilution method is shown in Figure 3. There was significant ($p<0.05$) reduction in cell number, and it was observed that the extract had high

(reduced the cell to 0.00±0.00 cfu/ml) bactericidal effects at 400 and 600 mg/mL on clinical and typed isolate of *Salmonella* Typhi. The bactericidal efficacy of the extract at 200 and 300 mg/ml concentration on clinical isolates were 283.11±1.37 and 84.04±0.55 cfu/ml whereas, on typed isolates is 234.66±0.34 and 71.31±0.95 cfu/ml respectively. Also, ciprofloxacin (5 mg/mL) had reduced the cell number to 19.62 (clinical isolate) and 18.31 cfu/ml (typed isolate) while the control group with DMSO had 439.91±0.53 and 449.11±1.42 x $10^3$ cfu/ml on clinical and typed isolates respectively.

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

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *G. hirsutum* extract are reported in Table 1. The MIC of extract against both isolates is 100 mg/ml while the MBC is 150 mg/mL.




| | 200 mg/ml | 300 mg/ml | 400 mg/ml | 600 mg/ml | DMSO | CPX (5 mg/ml) |
|---|---|---|---|---|---|---|
| Clinical | 283.11 | 84.05 | 0 | 0 | 439.91 | 19.62 |
| Typed | 234.66 | 71.31 | 0 | 0 | 449.11 | 18.31 |

**Figure 3: Comparative Bactericidal Effects of Clinical and Typed (ATCC 14028) Isolates of *Salmonella*Typhi to *G. hirsutum* Extract Using Broth Dilution assay**

CPX= Ciprofloxacin, DMSO= dimethylsulphoxide

**Table 1: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *G. hirsutum*Extract**

| ***Salmonella*Typhi Isolates** | **MIC (mg/mL)** | **MBC (mg/mL)** |
|---|---|---|
| Clinical | 100 | 150 |
| Typed | 100 | 150 |

MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration

**FTIR spectra of *G. hirsutum* extract**

The results presented in Figure 4 and Table 2 showed the FTIR spectra and spectral peak values and functional groups obtained for leaf extract of *G. hirsutum* respectively. Figure 4 revealed the peaks generated at different wavelengths ($cm^{-1}$) and Table 2 showed that there were eleven (11) different peaks generated which represents the following functional groups; 1,2,4-trisubstituted arenes, sulfoxide, vinyl ether, phenol, carboxylic acid, conjugated alkene, α, β-unsaturated ester, allene, allene, isothiocyanate and alcohol at wavenumber $\nu_{max}$ 879.7, 1043.7, 1200.2, 1326.9, 1394.0, 1617.7, 1714.6, 1912.1, 1986.7, 2087.3 and 3339.7 $cm^{-1}$ respectively.

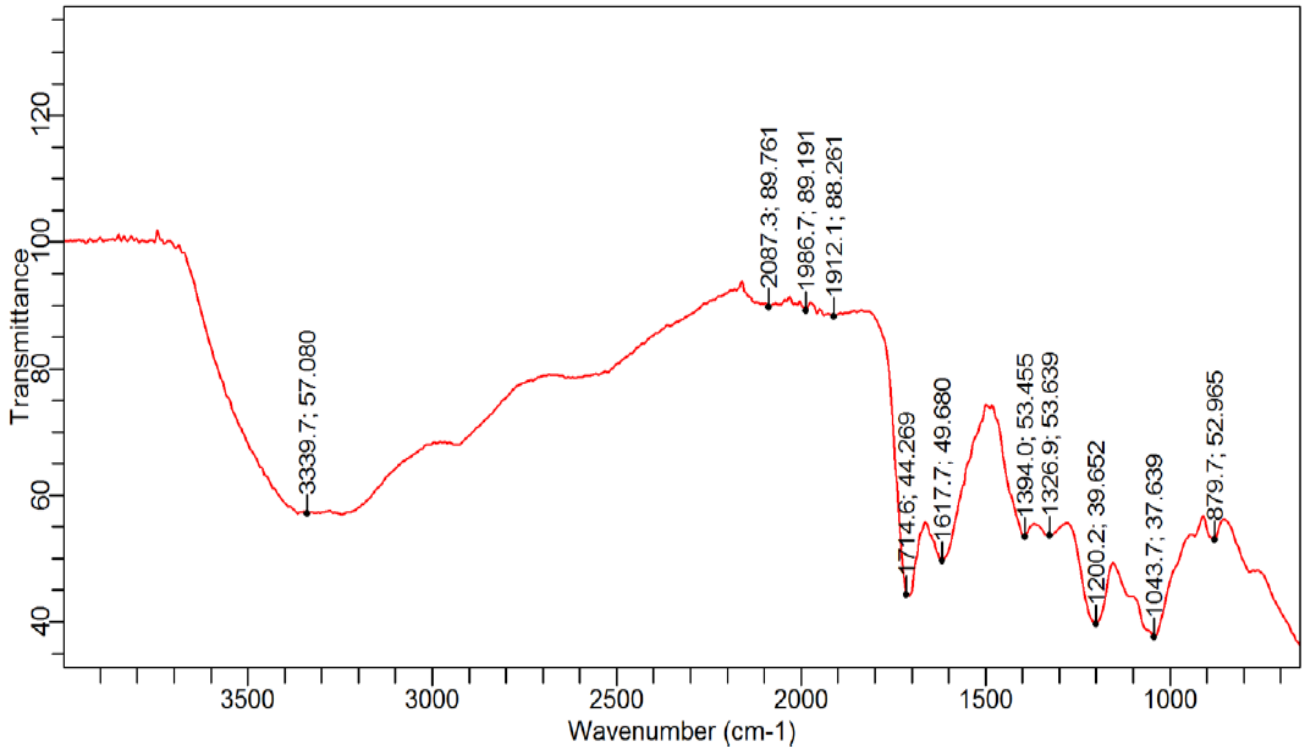



**Figure 4: FTIR spectra of *G. hirsutum* extract**

**Table 2: FTIR spectral peak values and functional groups obtained for leaf extract of *G. hirsutum***

| S.no | Peak values ($cm^{-1}$) | Functional group | Interpretation |
|---|---|---|---|
| 1 | 879.7 | C-H bending | 1,2,4-trisubstituted arenes |

| | | | |
|---|---|---|---|
| 2 | 1043.7 | S=O stretching | Sulfoxide |
| 3 | 1200.2 | C-O stretching | Vinyl ether |
| 4 | 1326.9 | O-H bending | Phenol |
| 5 | 1394.0 | O-H bending | Carboxylic acid |
| 6 | 1617.7 | C=C stretching | Conjugated alkene |
| 7 | 1714.6 | C=O stretching | α, β-unsaturated ester |
| 8 | 1912.1 | C=C=C stretching | Allene |
| 9 | 1986.7 | C=C=C stretching | Allene |
| 10 | 2087.3 | N=C=S stretching | Isothiocyanate |
| 11 | 3339.7 | O-H stretching | Alcohol |

C= Carbon, O= Oxygen, S= Sulphur, N= Nitrogen, H= Hydrogen

## DISCUSSION

Typhoid fever could cause serious health problems although it is treated with antibiotics, however the increase in resistance of *S. typhi* to conventional antibiotic therapy, has raised the need to search for alternative therapeutic methods of treatment (Kirk *et al.*, 2015).

Many organic solvents have been used as extraction solvent to assay antibacterial efficacy of different medicinal plants, however, it is a common practice among the 'Yoruba' tribe to extract bioactive components from leaf, root and stem of ethno-medicinal plants with lime juice. Based on information provided by both the traditional healer and the *in vitro* antibacterial test results, *in vivo* study shall be undertaken in a view to verifying the therapeutic efficacy of the extract. *In vivo* model could be employed for this study because it takes into account a possible prodrug effect and possible involvement of the immune system in the eradication of infections (Hilou *et al*., 2006).

*Salmonella typhi*, clinical and typed isolates had varying susceptibility to antibiotics used, ciprofloxacin inhibited clinical the isolates more than other antibiotics while chloramphenicol had least inhibition against clinical isolate. Comparative susceptibility patterns of clinical and typed (ATCC 14028) isolates of *Salmonella typhi* to *G. hirsutum* extract using agar well diffusion showed that the anti- *Salmonella* activity of the crude extract is concentration dependent and compare favourably with antibiotic (ciprofloxacin) using agar well diffusion and broth dilution method. This antimicrobial action may be due to the synergistic action of different chemical constituents, (Shahina *et al.*, 2007; Ogoti *et al.*, 2015; Marcelin *et al.*, 2016). The higher activity by the extract may be an indication that the phytoconstituents in the plant leaves are more in lime juice solvent than the organic solvent extracts (Marjorie, 1999; Omojasola and Awe, 2004). Presence of little traces of lime juice used for extraction could also be responsible for high antimicrobial efficacy. It has been reported that different phyto-constituents have different degrees of solubility in different types of solvents depending on their polarity. In a traditional setting, water is the solvent largely used to prepare these concoctions (Ologun *et al.*, 2019) but lime is being used occasionally.

The MIC of extract against both isolates is 100 mg/ml while the MBC is 150 mg/ml, the higher value of MBC than MIC indicates that the extract could have bacteriostatic effect at lower concentration and bactericidal at higher concentration. The finding of Cheesbrough (2014), stated that the level at which crude extracts inhibit test organisms is used to investigate the efficacy of chemotherapeutic agents under standard conditions.

This corroborates the finding of Omojasola and Awe (2004), which stated that the leaves extract of *Anacardium occidentale* and *Gossypium hirsutum* show antimicrobial activity against *Salmonella typhi*. The result of the antibacterial screening *in vitro* of *G. hirsutum* extracted with lime juice in this study justified the traditional use of this plant for the

treatment of gastroenteritis and other bacterial infections (Njau *et al*., 2014; Srinivasa and Narayanappa, 2015).

FTIR spectra showed that there were eleven (11) different peaks generated which represents the following functional groups; 1,2,4-trisubstituted arenes, sulfoxide vinyl ether, phenol, carboxylic acid, conjugated alkene, α, β-unsaturated ester, allene, allene, isothiocyanate and alcohol at different wavelength ($cm^{-1}$). The presence of sulfoxide, phenol, carboxylic acid and alcohol could be responsible for the high anti-*Salmonella* efficacy of the plant extract *in vitro.* The presences of sulfoxide group is unexpected in natural extracts, however, it may have originated from the lime juice used for the extraction. Sulfur compound has previously been reported in citrus (Shaw *et al*., 1980; Cannon *et al*., 2015).

## Conclusion

This study revealed that the extract of *G. hirsutum* proved more effective than ciprofloxacin when used *in vitro* and the efficacy is concentration dependent using agar well and broth dilution method. This justifies the acclaimed method of using lime juice for the extraction of bioactive components in medicinal plants traditionally. The study provides the basis for use of lime juice as solvent to extract the leaf of this plant in the development of drugs for management of typhoid fever.

## ETHICAL DISCLAIMER

Animal ethic Committee approval has been collected and preserved by the author.

## References

Anibijuwon I.I and Udeze O.A (2009).Antimicrobial activity of *Carica papaya* (Pawpaw Leaf) on some pathogenic organisms of clinical origin from South-Western Nigeria. *Ethnobotanical Leaflets*, **13**(7): 850-864.

Ao TT, Feasey NA, Gordon MA, Keddy KH, Angulo FJ, Crump JA. (2015) Global burden of invasive nontyphoidal Salmonella disease, 2010.*Emerg Infect Dis*.**21**(6):941–9

Ashokkumar, R.and Ramaswamy, M. (2014). Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian Medicinal plants. *International Journal of Current Microbiology and Applied Sciences*, **3**(1): 395-406.

Cannon R. J., Kazimierski A., Curto N. L., Li J., Trinnaman L., Janczuk A. J., Agyemang D. Da Costa N. C. and Chen M. Z. (2015). Identification, synthesis and characterization of Novel Sulfur-containing volatile compounds from the in-depth analysis of Lisbon lemon peels. *Journal of Agriculture and food chemistry*, **63**(7):1915-1931

Cheesbrough M. (2014). District Laboratory Practice in Tropical Countries. 2nd Ed. Cambridge University Press, United Kingdom, 480, 2014.

Egharevba RKA, Ikhatua MI. (2008). Ethnomedicinal uses of plants in the treatment of various skin diseases in Ovia North East, Edo State, Nigeria. Nigerian Tribune.;1-4.

Ene A. C., Atawodi, S. E. Ameh, D. A. Kwanashie, H. O. and Agomo, P. U. (2010). Locally used plants for malaria therapy amongst the Hausa, Yoruba and Ibo communities in Maduguri, Northern Nigeria. *Indian Journal of traditional Knowledge*, **9**(3): 486-490

Hilou A., Nacoulma G. and Guiguemde T. R. (2006).*In vivo* antimalarial activities of extracts from *Amaranthusspinosus* and *Boerhaaviaerecta* in mice.*Journal Ethnopharmacol*, **103**:236–240.

Kirk M. D, Pires S. M, Black R. E, Caipo M, Crump J. A, Devleesschauwer B, (2015). World Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal and viral diseases, 2010: a data synthesis. *PLoS Med*.**12**(100):19-21.

Kirk M. D, Pires S. M, Black R. E, Caipo M, Crump J. A, Devleesschauwer B, (2015). World Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal and viral diseases, 2010: a data synthesis. *PLoS Med*.**12**(100):19-21.

Kirk M. D, Pires S. M, Black R. E, Caipo M, Crump J. A, Devleesschauwer B, (2015). World Health Organization estimates of the global and regional disease burden of 22

foodborne bacterial, protozoal and viral diseases, 2010: a data synthesis. *PLoS Med*.**12**(100):19-21.

Kraft, K. (2009). Complementary/Alternative Medicine in the context of prevention of disease and maintenance of health. *Prev Med.* 2009 May 22.

Marcelin U., Bharathidasan R. and Prince L. (2016). Anti*salmonella* Activity of Selected Medicinal Plants.*World Journal of Pharmacy and Pharmaceutical Sciences,* **5**(4): 2512-2519

Marcelin U., Bharathidasan R. and Prince L. (2016). Anti*salmonella* Activity of Selected Medicinal Plants. *World Journal of Pharmacy and Pharmaceutical Sciences,* **5**(4): 2512-2519

Marjorie M.C. (1999). Plant products as antimicrobial agents. *Clinical Microbiology and Review,***12**(4):564-582.

Modi A. A., Wright E. C. and Seeff L. B. (2007). Complementary and Alternative Medicine (CAM) for the treatment of chronic Hepatitis B and C: a review. *Antiviral Therapy*.**12**(3):285-95.

Moquin B., Blackman M. R., Mitty E. and Flores S. (2009).Complementary and Alternative Medicine (CAM).*Geriatr Nursery,* **3**:196-203.

Njau, E.A., Alcron, J, Ndakidemi, P., Chirino-Trejo, M and Buza, J, (2014). Antimicrobial and antioxidant activity of crude extracts of *Rauwolfia caffra var. caffra* (Apocynaceae) from Tanzania. *International Journal of Biology*. **6** (4): 156.

Ogoti P.,Magiri E., Magoma G., Kariuki D. and Bii C. (2015). In vitro anti-Salmonella activity of extracts from selected Kenyan medicinal plants. *Journal of Medicinal Plants Research*, **9**(8): 254-261

Ogoti P.,Magiri E., Magoma G., Kariuki D. and Bii C. (2015). *In-vitro* anti-Salmonella activity of extracts from selected Kenyan medicinal plants. *Journal of Medicinal Plants Research*, **9**(8): 254-261

Ologun, O., Dada, E. O. and Ajayi, K. O. (2019).*In vitro* Study on Anti-salmonella Activities of *Boerhaavia diffusa* (L. syn) Leaf Extract. *International Journal of Pathogen Research,* **3**(1): 1-10

Omojasola, P. F. and Awe, S. (2004). The antibacterial activity of the leaf extracts of the leaf extracts of *Anacardium occidentale* and *Gossypium hirsutum* against some selected microorganisms. *Bioscience Research Communications*, **60**(1): 25-58.

Omoya, F. O. Momoh, A. O. and Olaifa, A. O. (2015). *In vivo* Effect of Cassava Flakes Mixed with *Euphorbia heterophylla* against *Salmonella typhi. European Journal of Medicinal Plants*, 7(1): 38-45

Shahina N., Samia A. and Sheikh A. R (2007). *In-vitro* antimicrobial activity of the extracts derived from *Terminaliacatappa. Res. Journal Microbiology*, **2**(2): 180-184.

Shaw P. E., Ammons J. M. and Braman R. S. (1980). Volatile sulfur compounds in fresh orange and grape fruit juice: identification, quantification and possible importance to juice flavor. *Journal of Agriculture and food chemistry*, **28**(4): 778-781

Srinivasa, M.K.M and Narayanappa, M (2015).*In-vitro* study of antibacterial activity of leaf and root Extract of *Rauwolfia serpentina* against Gram positive and Negative bacterial strains. *International Journal of Recent Research in Interdisciplinary Sciences*. **2** (3):33-37.

Zhang, X., Jeza, V. T., and Pan, Q. (2008).*Salmonella Typhi*: from a Human Pathogen to a Vaccine Vector. *Cellular and Molecular Immunology*, **5**(2):91-99