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

ABSTRACT

Invasive *Salmonella* infections are responsible for a significant burden of morbidity and mortality worldwide and with the increase in resistance to anti-typhoid, medicinal plants have gained popularity among both urban and rural dwellers in the treatment of the ailment. The present study was undertaken to investigate anti-*Salmonella* activity of *Gossypium hirsutum* leaf extract on *Salmonella typhi* (clinical isolate) using fresh lime juice as extraction solvent. Extraction lime juice and bioactive components of the plant leaf and *in vitro* anti-*Salmonella* activity of extract were carried out using standard microbiological methods while *Salmonella typhi* ATCC 14028 (Type isolate) was used as control. Fourier Transform Infrared Spectrophotometer (FTIR) was used to assay the functional groups in the extract. The result revealed that clinical (35.10±0.45 mm) and typed (36.71±0.32 mm) isolates had highest susceptibility to ciprofloxacin while the crude extract showed inhibition against *Salmonella* with zone of inhibition range 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 isolate at 600 and 200 mg/ml respectively. Fourier Transform Infrared Spectrophotometer (FTIR) revealed different functional groups in the extract which are 1,2,4-trisubstituted, sulfoxide, vinyl ether, phenol, carboxylic acid, conjugated alkene, α, β-unsaturated ester, allene and alcohol. The overall results indicate that the lime juice extract of *G. hirsutum* has the potential to provide an effective treatment for salmonellosis, including typhoid fever. However, it is necessary to ascertain the safety of this extract and extrapolate these results in large animals, in further studies.

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

Introduction

Salmonella is the causative agent of salmonellosis. It is a rod-shaped gram-negative facultative anaerobe bacterium belonging to the *Enterobacteriaceae* family. Among more than 2,300 closely-related *Salmonella* serovars recognized, *Salmonella typhi* and Paratyphi are pathogenic exclusively for humans, and cause systemic infections and typhoid fever, whereas others such as *S. Typhimurium* cause gastroenteritis (Zhang *et al.,* 2008; Kirk *et al.,* 2015). Salmonellosis is more prevalent in developing parts of the world in Africa, Asia, and South America. South Asia are at highest risk for infections that are nalidixic acid-resistant or multidrug-resistant (i.e., resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole). In humans, salmonellosis is seen in two kinds of 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*, and there are estimated 20 million cases and 200,000 deaths worldwide each year (Ao *et al.*, 2015).

Typhoid fever is a serious problem. Although it is treated with antibiotics, however because of the increase in resistance of the aetiologic cause, *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, 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 our previous study on anti*Salmonella* activity of *Euphorbia heterophylla* aqueous extract and cassava flakes (white and yellow), the combinations of *Euphorbia heterophylla* + white cassava flakes had the highest anti*Salmonella* activity *in-vitro* while the result of the histopathological studies *in-vivo* showed that *Euphorbia heterophylla* mixed with yellow and white cassava flakes can be used in the control of *Salmonella typhi* infections, especially the problems caused in the liver and kidney by the organism. It can be used to treat disruptions in the kidney and liver with mild histopathological features on liver, heart and kidney of mice compared with those that were infected and not tra therapeutic agent being used for treating the infection (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 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. There has not been any published literature on extraction of plant and examination of phytochemicals using FTIR with lime as extraction solvent, therefore this study is a baseline study for further studies.

Materials and methods

Collection of leaves of *Gossypium hirsutum*

Fresh leaves of *Gossypium hirsutum* were collected before the sunrise to prevent plant photo-oxidation from the North gate at Federal University of Technology, Akure, the leaf that has no injury nor chlorosis were sorted out and kept in a clean sack for further work and identified by the expert in the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure Ondo State. The plant is popularly called "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 so that any dirt or microorganism residing on the surface will not be transferred to the fruit's interior part. 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

The method of Tomassini *et al*. (2009) was used. 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 (3 days) 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 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 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 prepared according to method of 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 commercial antibiotics discs (Fondoz Laboratories Ltd, Nigeria) used were; 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.

Broth dilution test

Also, effects of extract on anti-*Salmonella* efficacy of the extract 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 it 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.

Determination 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

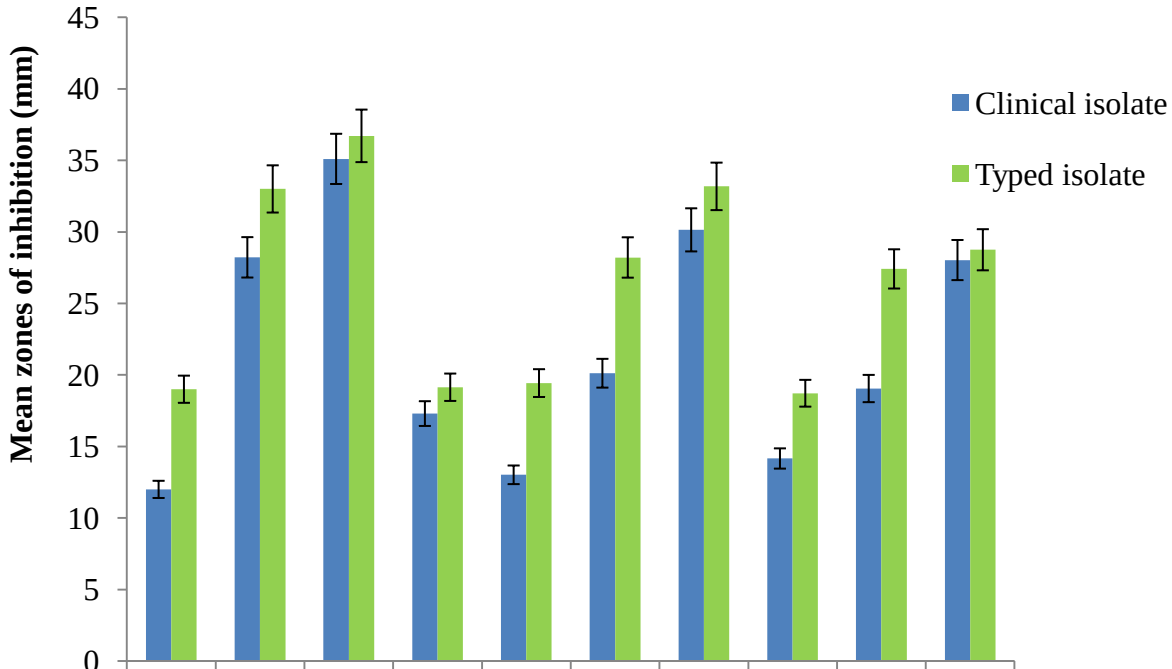
Comparative Antibiotic Susceptibility Patterns of Clinical and Typed (ATCC 14028) Isolates of *Salmonella*Typhi

The result showed in 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 had varying susceptibility to antibiotics used, ciprofloxacin inhibited clinical (35.10±0.45 mm) and typed (36.71±0.32 mm) isolates more than other antibiotics and there was no significant ($p<0.05$) difference between their zones of inhibition to 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.

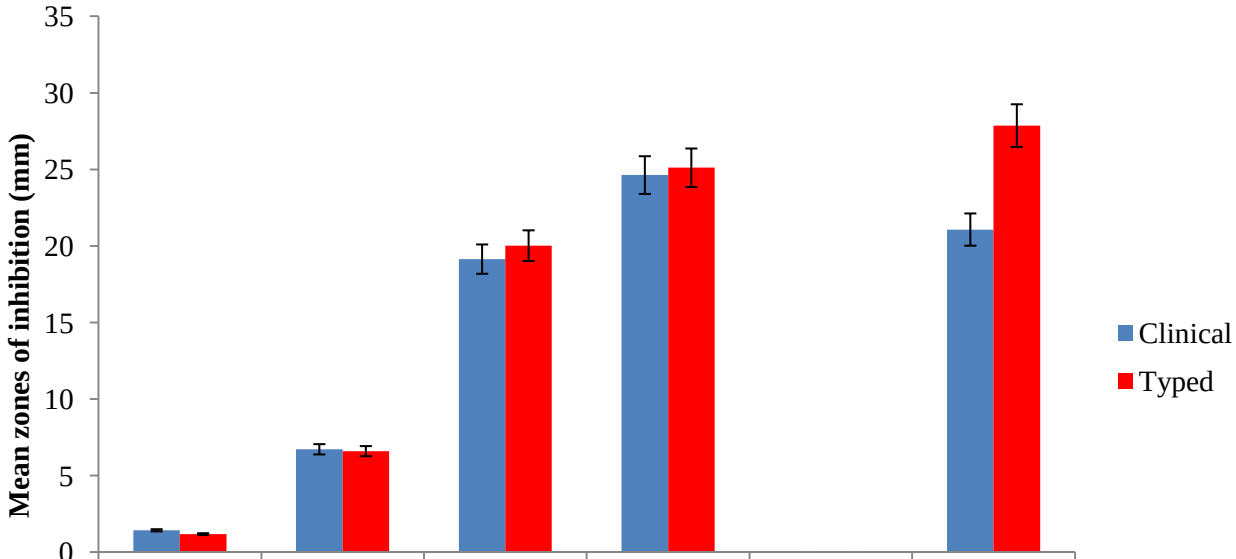


	CHL	ZIN	CPX	AMX	AMP	GEN	PFX	TET	STR	SP
Clinical isolate	12	28.22	35.1	17.3	13.02	20.12	30.14	14.16	19.05	28.03
Typed isolate	19	33	36.71	19.14	19.43	28.21	33.18	18.72	27.41	28.75

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

KEYS:

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

Keys:

CPX= Ciprofloxacin, DMSO= dimethylsulphoxide

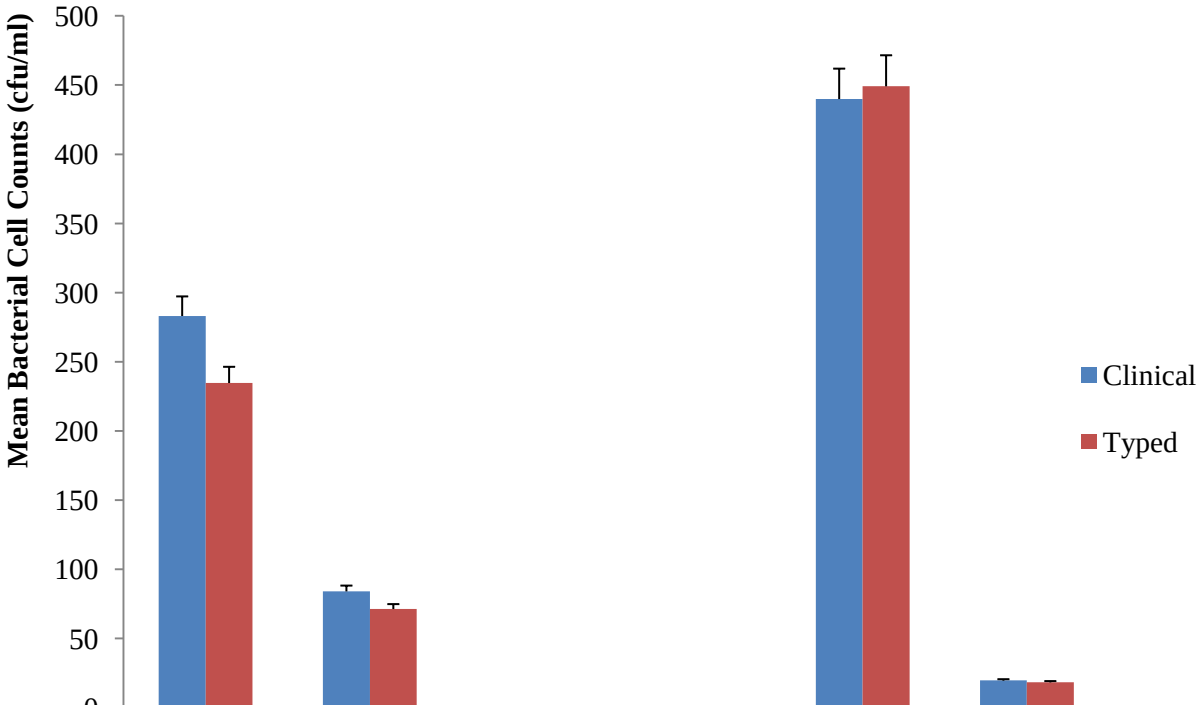
Comparative Bactericidal Effects of *G. hirsutum* Extract on Clinical and Typed (ATCC 14028) Isolates of *Salmonella*Typhi Using Broth Dilution

Comparative bactericidal effects of *G. hirsutum* extract on clinical and Typed (ATCC 14028) isolates of *Salmonella*Typhi using broth dilution method is revealed 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±0.95cfu/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 10^3cfu/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

Keys:

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

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

Fourier Transform Infrared Spectrophotometer (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, sulfoxide, vinyl ether, phenol, carboxylic acid, conjugated alkene, α, β-unsaturated ester, allene, allene, allene and alcohol at wavelength 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.

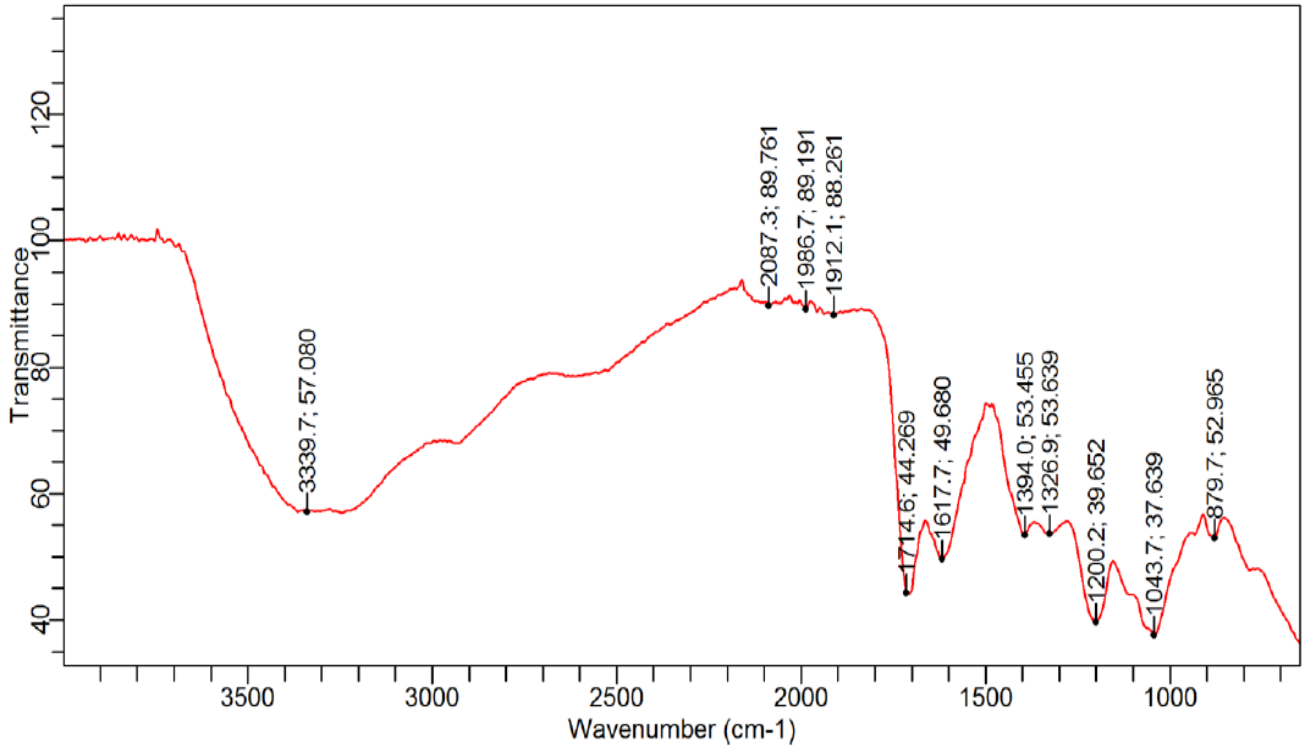


Figure 4: Fourier Transform Infrared Spectrophotometer (FTIR) spectra of *G. hirsutum* extract

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

254 ***hirsutum***

S.no	Peak values (cm^{-1})	Functional group	Interpretation
1	879.7	C-H bending	1,2,4-trisubstituted
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	C=C=C stretching	Allene
11	3339.7	O-H stretching	Alcohol

255

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

257 **DISCUSSION**

258 Typhoid fever is a serious problem. Although it is treated with antibiotics, however because
259 of the increase in resistance of the aetiologic cause, *S. typhi* to conventional antibiotic
260 therapy, there is a need to search for alternative therapeutic methods of treatment (Kirk *et al.*,
261 2015).

262 Many organic solvents have been used as extraction solvent to assay antibacterial efficacy of
263 different medicinal plants, however, it is commonly practice among the 'Yoruba' tribe to
264 extract bioactive components of leaf, root and stem of ethno-medicinal plants with lime juice.
265 Based on information provided by both the traditional healer and the *in vitro* antibacterial test

results, *in vivo* study was undertaken in a view to verifying the therapeutic efficacy of the extract. An *in vivo* model was employed for this study because it takes into account a possible prodrug effect and possible involvement of the immune system in the eradication of an infection (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, some of which probably are last upon extraction with solvent (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 in lime than the organic solvent in the previous report (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 justifies the traditional use of this plant as medicinal 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, sulfoxide, vinyl ether, phenol, carboxylic acid, conjugated alkene, α, β-unsaturated ester, allene, allene, allene and alcohol at different wavelength (cm^{-1}). The presence of sulfoxide, phenol, carboxylic acid and alcohol could responsible for the high anti*Salmonella* efficacy of the plant extract *in vivo* and *in vitro.*

Conclusion

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. 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.

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.

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.

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