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

ABSTRACT

Blue = deletion

Red = inclusion

Invasive *Salmonella* infections are responsible for a significant burden of morbidity and mortality worldwide. and with the There has been increase in resistance to anti-typhoid prescription drugs and the medicinal plants have gained popularity among both urban and rural dwellers in the treatment of not only typhoid fevers but also to treat various the 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, and bioactive components of the plant leaf and *in vitro* anti-*Salmonella* activity of extract were all carried out using standard microbiological methods. while *Salmonella typhi* ATCC 14028 (Type isolates) was used as control. Fourier Transform Infrared Spectrophotometer (FTIR) was used to assay identify the functional groups in the extract. The result revealed that clinical (35.10±0.45 mm) is it diameter? Specify. and typed (36.71±0.32 mm) isolates had showed highest susceptibility to ciprofloxacin. while tThe crude extract showed an inhibition against *Salmonella* with zone of inhibition range 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/mlL, respectively. Fourier Transform Infrared Spectrophotometer (FTIR) revealed different indicated the presence of various functional groups in the extract which are such as 1,2,4-trisubstituted, sulfoxide, vinyl ether, phenol, carboxylic acid, conjugated alkene, α, β-unsaturated ester, allene and alcohol. The overall results indicated that the lime juice extract obtained from of *G. hirsutum* leaves using a lime juice has the potential for to provide an effective treatment for of salmonellosis, including typhoid fevers. However, it is necessary to ascertain the safety of this extract and extrapolate these results in large animals, (what do you mean by large animals?) in further studies.

Modifye this underlined sentence

Key words: *Salmonella,* lime juice, crude extract, functional group (include a few more strong key words)

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 more than 2,300 closely-related *Salmonella* serovars recognized, *Salmonella typhi* and Paratyphi

(the latter one should be italics) 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).

The above underlined sentence is not clear. Simplify the sentence.

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

South Asia are is at highest risk for infections that are nalidixic acid-resistant or multidrug-resistant (i.e., resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole).

The above sentence is not clear.

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 tThere are estimated 20 million cases and 200,000 deaths worldwide have been reported each year (Ao *et al.*, 2015).

Typhoid fever is a serious problem. Although it is could be treated with antibiotics, however because of the due to 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).

The above sentence is not clear.

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 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.

Simplify the above sentence and rewrite

It (specify) 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 (what is this?) 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) why different font? hHowever, 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. There has not been any previous 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.

Modify the sentence

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 around the North gate at of Federal University of Technology, Akure. tThe leaf leaves that has no injury nor or chlorosis were sorted out and kept in a clean sack for further work. and The plant material was identified by the an expert (specify the name and designation) in the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure Ondo State. The vernacular name of this 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

Plant extract was prepared as per the method reported in the literature (Tomassini *et al.* 2009). 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 the ratio of 1:10 each (i.e. 50 g of the powdered sample in 500 mlL 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 (specify the name) 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 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 following commercial antibiotics discs (Fondoz Laboratories Ltd, Nigeria) were 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.

(Is the test conducted in duplicate or triplicate?)

Change throughout from mg/ml to mg/mL

Broth dilution test

Also, The 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).

Modify the sentence

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.

Change throughout from mg/ml to mg/mL

Determination 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 (specify name and designation) 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

specify in which assay?

The results 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 (space required) clinical and typed isolates had exhibited 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.

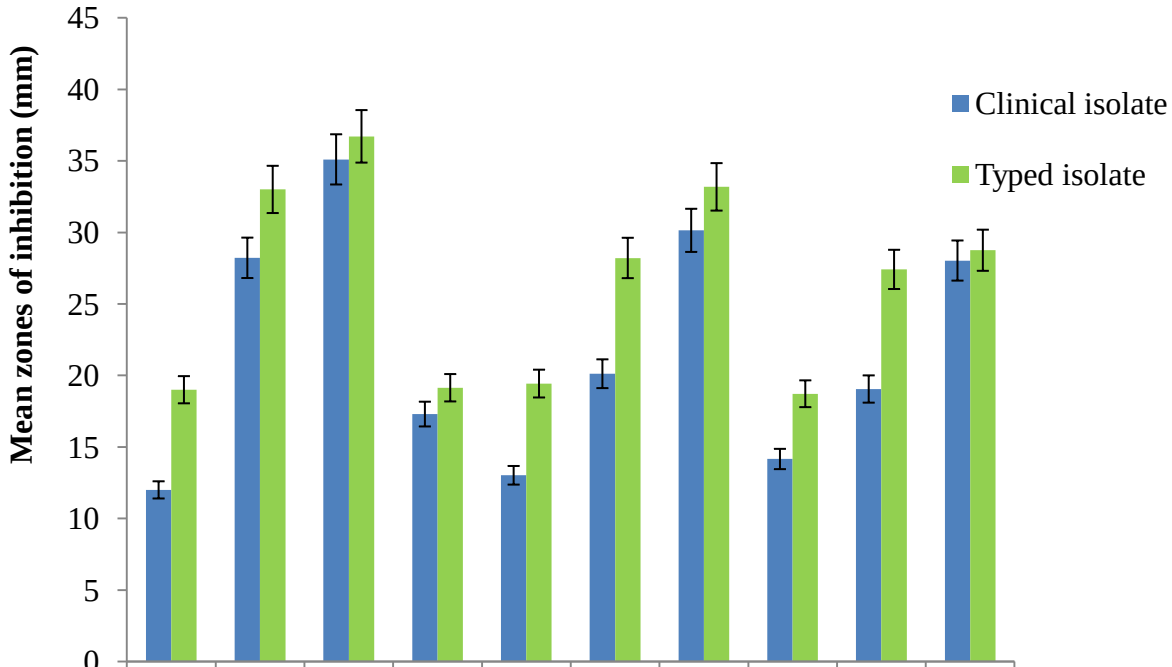
Simplify and modify the sentence

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.

Change throughout from mg/ml to mg/mL



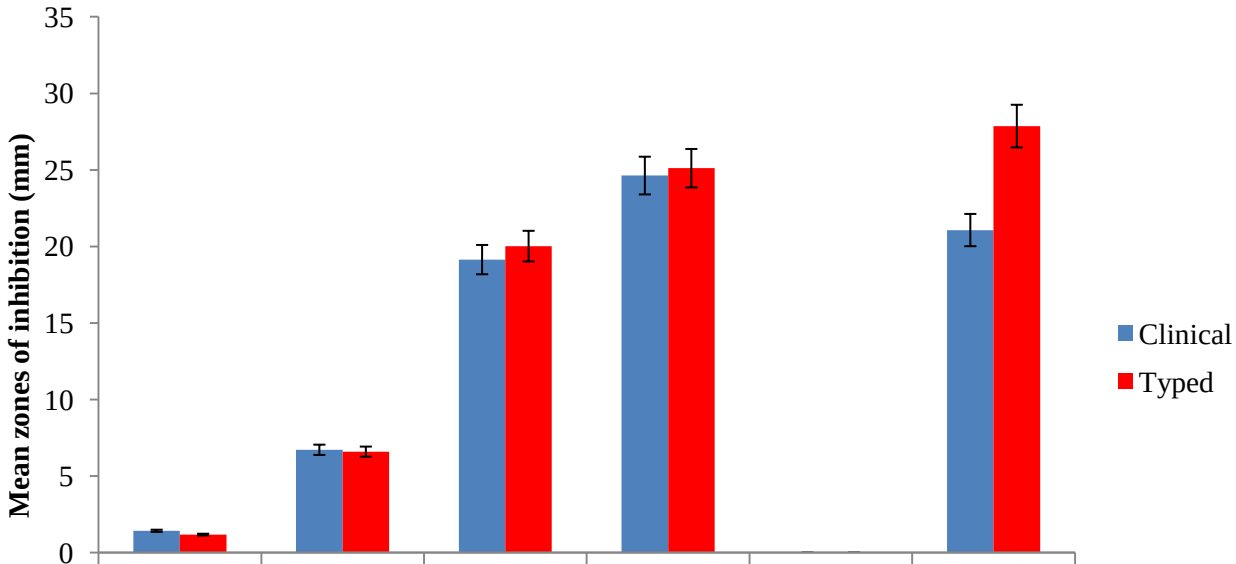
	CHL	ZIN	CPX	AMX	AMP	GEN	PFX	TET	STR	SP
Clinical isolate	12	28.22	35.1	17.3	13.02	20.12	30.14	14.16	19.05	28.03
Typed isolate	19	33	36.71	19.14	19.43	28.21	33.18	18.72	27.41	28.75

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

specify in which assay?

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 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

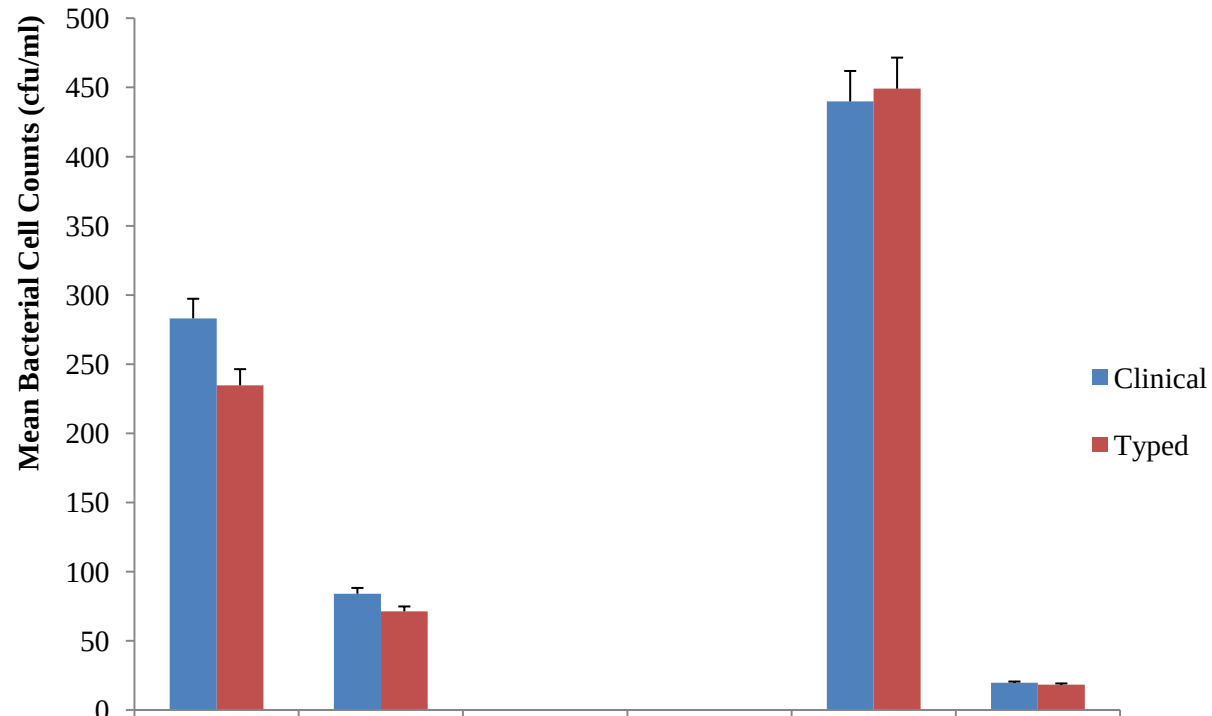
clinical and typed isolate of *Salmonella*Typhi. The bactericidal efficacy of the extract at 200 and 300 mg/ml concentration on clinical isolates were 283.11±1.37 and 84.04±0.55 cfu/ml whereas, on typed isolates is 234.66±0.34 and 71.31±0.95cfu/ml respectively. Also, ciprofloxacin (5 mg/ml) had reduced 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.

Change throughout from mg/ml to mg/mL

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.

Change throughout from mg/ml to 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

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

Change throughout from mg/ml to mg/mL

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 wavenumber ν_{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.

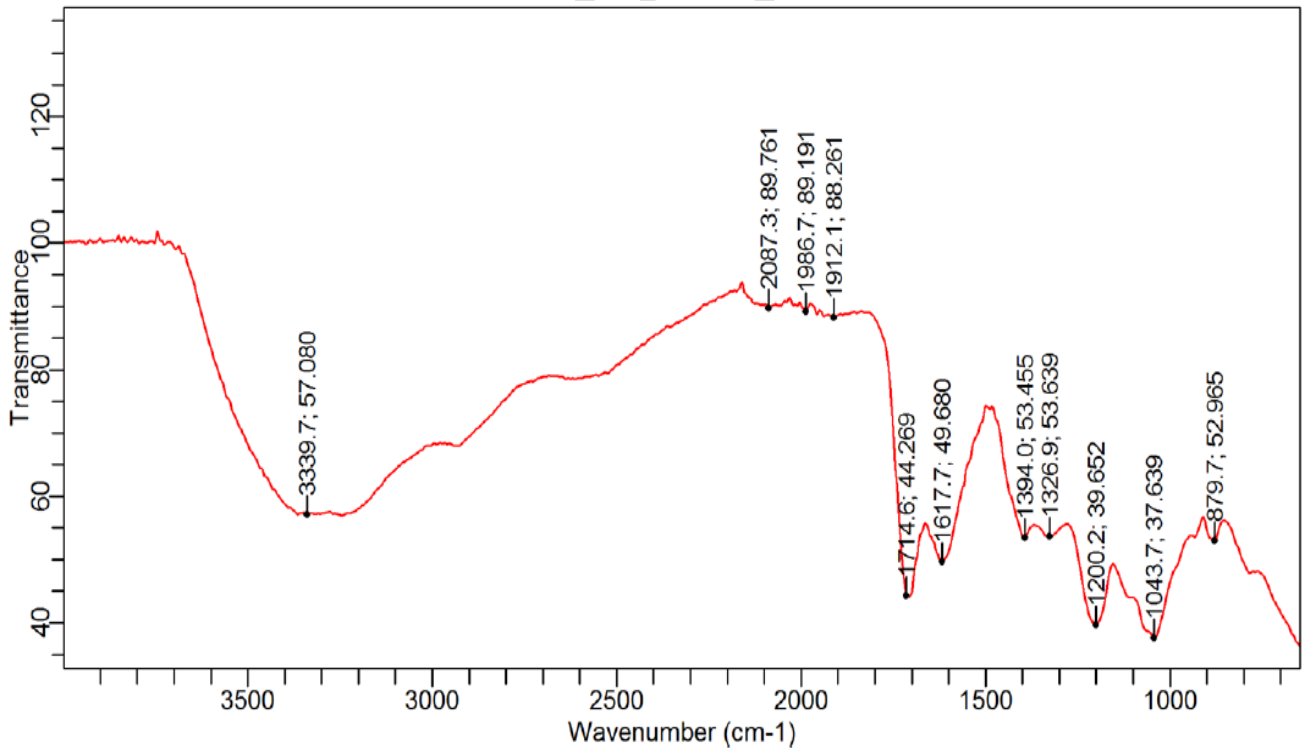


Figure 4: Fourier Transform Infrared Spectrophotometer FTIR spectra of *G. hirsutum* extract

The appearance of this spectrum is not good. Baseline corrections should be done. The finger print region (900-1400cm^{-1}) alone should have many unassigned peaks. Particularly, for crude extract, this region should be much more complex. Based on the appearance of this spectrum, we shall not give any importance or credit to the this plant *G. hirsutum* extract Because, almost, any crude extract from any plant will typically have all these peaks.

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 of what?
2	1043.7	S=O stretching	Sulfoxide (Sulphur containing compounds in natural products are very rare) (The authors should report any previously reported papers on this plant has Sulphur containing compounds)
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 (this value should be around 1950 cm^{-1} and not 2087.3 cm^{-1})
11	3339.7	O-H stretching	Alcohol

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

DISCUSSION

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).

Modify the above sentence.

Many organic solvents have been used as extraction solvent to assay antibacterial efficacy of different medicinal plants, however, it is a commonly practice among the 'Yoruba' tribe to extract bioactive components of 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 was shall be undertaken in a view to verifying the therapeutic efficacy of the extract. An *in vivo* model was 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 an 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 Aantimicrobial 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 more in limejuice solvent than the organic solvent extracts 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 justifiedes 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 of what?, sulfoxide (need supportive evidence) 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 be responsible for the high anti*Salmonella* efficacy of the plant extract *in vivo* and *in vitro.* (why not the other chemicals?)

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. Put this sentence as a last sentence of this paragraph. 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.

Summarise the results obtained and modify this paragraph.

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