Activity of Semi Purified Fractions of *T. diversifolia* and *W.ugandensis* against Selected Clinical Isolates of *Salmonella* Strains

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Abstract: The aim of this study was to determine *in vitro* anti-*Salmonella* activity of semi purified fractions of methanol extract of *Tithonia diversifolia* leaves, ethyl acetate and hexane extracts of *Warburgia ugandensis* stem bark and roots against four clinical isolates of *Salmonella* strains. The methanol, ethyl acetate and hexane extracts of the two plants were purified using silica column chromatography. Minimum Inhibitory Concentrations (MICs) of the semi purified fractions determined by microdilution assay. The MIC values of the fractions ranged from 1.22-312.5µg/ml. These results were comparable with that of ciprofloxacin (1.22-19.53µg/ml). Gas Chromatography-Mass Spectrometry (GC-MS) analysis was carried out to identify the important compounds in the active fractions. A total of thirty three known compounds were identified by GC-MS analysis. For example, hexadecanoic acid, 9, 12-octadecadienoic acid (Z, Z), 1, 2-benzenedicarboxylic acid and beta-sesquiphellandrene identified by GC-MS are known to have antimicrobial property. These findings demonstrate that the semi purified fractions of *T.diversifolia* and *W. ugandensis* are diverse and exhibit appreciable amount of anti-*Salmonella* activity and thus have great potential as a source for natural health products.
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1. Introduction

Salmonella serotype Typhimurium (*S.* ser. Typhimurium), is a Gram-negative bacterial pathogen that infects humans and animals, causing significant morbidity and mortality worldwide (Fink and Cookson, 2007). It is an obligate intracellular bacterial pathogen that causes gastroenteritis in millions of people worldwide each year (Grassl, *et al*., 2008). For instance, the Centre for Disease Control (CDC) estimates that there are nearly 1.4 million food-borne *Salmonella* infections annually in the USA (Mead, *et al*., 1999). Various strategies have been employed in the treatment and management of *Salmonella* infections.

Fluoroquinolones and tetracyclines are most commonly used to treat *Salmonella* infections. However, *Salmonella* strains resistant to these antibiotics have been reported in Korea and other countries (Choi *et al*., 2005, and Stevenson *et al*., 2007). One major concern to public health has been the global dissemination of *S.*typhimurium Definitive Type 104, which is resistant to cotrimoxazole, nalidixic acid and ampicilin (Perron *et al*., 2008, Kariuki *et al*., 2010). The rise in antibiotic-resistant strains has led to increased interest in use of plant materials to develop new effective drugs. Hence, there is a need for scientific evidence based validation of bioactive phytochemicals (Adeniyi and Ayepola, 2008; Karim *et al*., 2011).

Plants used in this study have traditionally been associated with disease curative and preventive practices in many countries for a long time. Garcia and Delgado, (2006), have reported that *Tithornia diversifolia* has promising medicinal value. Skin products formulated from *T. diversifolia* extracts have been shown to have antimicrobial properties (Kareru *et al*., 2010).

In Ethiopia *Warburgia ugandensis* extracts are used to treat malaria, tuberculosis, bronchitis, pneumonia, hepatitis, tapeworm, gonorrhea, and asthma (Wube *et al*., 2010, Were *et al*., 2010 and Opiyo *et al*., 2011).

In the present study, *in vitro* bioassay guided purification of anti-*Salmonella* compounds from leaf of *T. diversifolia*; stem bark and root of *W. ugandensis* were carried out. The active fractions were identified by Gas Chromatography-mass spectrometry (GC-MS) analysis.

2. Materials and Methods

2.1 Salmonella strains

Clinical samples of *S.*ser.Typhi (ATCC 13347), *S.*ser.Typhi (ATCC 43579), *S.enterica* (ATCC 2162) and *S.* ser. Typhimurium (ATCC 1408), were provided by the Centre of Microbiology Research, Kenya Medical Research Institute (CMR-KEMRI) for this study.

2.2 Plant Materials

Methanol extract of *T.diversifolia* leaf, ethyl acetate and hexane extracts of stem bark and root respectively of *W.ugandensis* were obtained from the previous research work (Ogoti *et al*., 2015).

2.3 Controls

Acetone was used as negative control and Ciprofloxacin (Transchem pharmaceutical Ltd, Kenya) was used as positive control.

2.4 Silica gel column chromatography

A 60 cm long glass column with the diameter of 10 cm was filled with 1.5 kg of silica gel, mesh size 60-120. Methanol extracts of *T.diversifolia* leaf, ethyl acetate extracts of *W.ugandensis* bark and hexane extracts of *W.ugandensis* root were subjected separately to column chromatography in silica gel glass column. The column was eluted with hexane followed by hexane-ethyl acetate at increasing polarity. Fifty methanol, 20 ethyl acetate and 100 hexane fractions of 50ml each were collected, analyzed on TLC (Merck, S 0,032-0,063mm) with dichloromethane, chloroform and ethyl acetate solvents (3:2:1). The spots with similar RF values of methanol, ethyl acetate and hexane fractions were pooled to give 7, 4 and 7 sub-fractions of *T.diversifolia* leaf, *W.ugandensis* stem bark and root respectively. Various sub-fractions collected and labeled as documented in the Table 1, 2 and 3. All the obtained sub-fractions were collected in sample vials and stored at -20°C.

2.5 Minimum inhibitory concentration (MIC) values of semi purified fractions

The MIC values were determined using microdilution assay as described by Eloff (1998). Ciprofloxacin was used as positive controls and acetone was used as negative control. Plant fractions were tested against *Salmonella* strains with varying concentration ranging from 2.5mg/ml-0.0012mg/ml. Briefly, 100 µl of sterile distilled water was added to each well of 96-well microtitre plates (SIGMA Aldrich, German) followed by the addition of 100 µl of 2.5mg/ml and thereafter serially diluted plant fractions. Then 100 µl of *Salmonella* strains were added to each micro well to give a final volume of 200. The prepared plates were sealed to avoid drying and incubated overnight at 37□C. After overnight incubation, 50µl of 5mg/ml 2, 3, 5 Triphenyltetrazolium chloride (SIGMA Aldrich, German) was added to the wells and incubated overnight. The pink colour was indicative of bacterial growth while lack of color was linked to growth inhibition. The MIC was defined as the lowest concentration of plant fraction that completely suppresses the growth of *Salmonella* strains.

2.6 Gas Chromatography Mass Spectra

Gas chromatography-MS analysis GC-MS analysis was performed in Jomo Kenyatta University of Agriculture and Technology, Juja, Kenya. About 2 ml of methanol, ethyl acetate and hexane fractions were subjected to GC-MS analysis using CE GC 8000 top MSMD 8000 Fyson instrument with Db 35 mr column (10 m x 0.5 mm, 0.25 mm film thickness). Analysis was done at between 100-250ºC for 3 minutes a flow rate maintained at 1ml/min in the split mode (1:50) (An aliquot (2 ml) of oil was injected into the column with the injector heater at 250ºC). Analytical conditions Injection temperature at 250ºC, interface temperature at 200ºC, quadruple temperature at 150ºC and ion source temperature at 230ºC were maintained.

2.7 Identification of major components

The mass spectra of compounds in samples were obtained by electron ionization (El) at 70 eV, and the detector operated in scan mode from 20 to 600 atomic mass units (amu). Identification was based on the molecular structure, molecular mass and calculated fragments. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST library version (2005), software, Turbomas 5.2.

2.8 Statistical Analysis

Anti-*Salmonella* activity was determined from means of duplicates in MICs. Collected data was analysed statistically using one way ANOVA (SAS, Version 9.0). Difference in values at $P<0.0001$ were considered statistically significant.

3. Results

3.1 Chromatographic fractionation of plant extracts

Methanol extracts of *T.diversifolia* leaf, ethyl acetate extracts of *W.ugandensis* bark and hexane extracts of *W.ugandensis* root were fractionated separately on silica column. Seven methanol fractions of *T.diversifolia* leaf designated T_A, T_B, T_C, T_D, T_E, T_F and T_G were obtained after silica TLC analysis. Likewise four ethyl acetate fractions of *W.ugandensis* bark designated E_A, E_B, E_C and E_D were determined by silica TLC analysis. Meanwhile, seven hexane fractions of *W.ugandensis* root designated H_A, H_B, H_C, H_D, H_E, H_F and H_G were also obtained by TLC analysis. All fractions were evaluated for anti-*Salmonella* activity by microdilution assay.

3.2 Anti-*Salmonella* activity of semi purified fractions of plant extracts

Eighteen semi purified fractions from methanol extracts of *T.diversifolia* leaves, ethyl acetate stem bark and hexane root extracts of *W.ugandensis* were screened for anti-*Salmonella* activity against 4 clinical isolates of *Salmonella* strains; *S.*ser.Typhi (ATCC 13347), *S.*ser.Typhi (ATCC 43579), *S.enterica* (ATCC 2162) and *S.* ser. Typhimurium (ATCC 1408) using microdilution assay.

The seven methanol fractions (T_A, T_B, T_C, T_D, T_E, T_F, T_G) had Minimum inhibitory concentration (MIC) values in the range of 1.22-312.5µg/ml, for the 4 clinical isolates of *Salmonella* strains. Table 1 shows MIC values of methanol fractions against the selected clinical isolates.

Table 1. MIC (µg/ml) of methanol fractions of *T.diversifolia* leaf

	Clinical isolates of *Salmonella* strains			
Plant fractions	***S.* ser. Typhimurium (ATCC 1408)**	***S.*ser.Typhi (ATCC 13347)**	***S.*ser.Typhi (ATCC 43579)**	***S.enterica* (ATCC 2162)**
T_A	9.77^{d}	2.44^{d}	4.88^{d}	4.88^{d}
T_B	312.5^{a}	1.22^{d}	19.53^{d}	1.22^{d}
T_C	78.13^{cd}	1.22^{d}	78.13^{cd}	1.22^{d}
T_D	39.06^{d}	1.22^{d}	19.53^{d}	39.06^{d}
T_E	39.06^{d}	1.22^{d}	19.53^{d}	39.06^{d}
T_F	39.06^{d}	1.22^{d}	78.13^{cd}	312.5^{a}
T_G	19.53^{d}	4.88^{d}	78.13^{cd}	2.44^{d}
CIPRO	19.53^{d}	1.22^{d}	9.77^{d}	1.22^{d}
Acetone	ND	ND	ND	ND

T: *T.diversifolia* leaf, T_A: Combined fractions1-10, T_B: Combined fractions 11-17, T_C: Combined fractions 18-24, T_D: Combined fractions 25-32, T_E: Combined fractions 33-39, T_F: Combined fractions 40-44 and T_G: Combined fractions 45-50, CIPRO: Ciprofloxacin (Positive control) Acetone: Negative control, ND: Not determined. Values are means of duplicate reading. Means followed by different superscript letters in the table above are significantly different at P<0.0001.

Table 2 shows four ethyl acetate fractions with MIC values ranged from 1.22 to 312.5 µg/ml against the clinical isolates of *Salmonella* strains. All the fractions tested had anti-*Salmonella* activity.

Table 2. MIC (µg/ml) of fractions of ethyl acetate extracts of *W.ugandensis* stem bark

Plant Fractions	**Clinical isolates of *Salmonella* strains**			
	***S.* ser. Typhimurium (ATCC 1408)**	***S.*ser.Typhi (ATCC 13347)**	***S.*ser.Typhi (ATCC 43579)**	***S.enterica* (ATCC 2162)**
E_A	156.25^{bc}	19.53^{d}	156.25^{bc}	156.25^{bc}
E_B	9.77^{d}	2.44^{d}	312.5^{a}	9.77^{d}
E_C	1.22^{d}	1.22^{d}	19.53^{d}	1.22^{d}
E_D	1.22^{d}	1.22^{d}	9.77^{d}	1.22^{d}
CIPRO	19.53^{d}	1.22^{d}	9.77^{d}	1.22^{d}
Acetone	ND	ND	ND	ND

E: Ethyl acetate *W.ugandensis* stem bark, E_A: Combined fractions 1-6, E_B: Combined fractions 7-11, E_C: Combined fractions 12-16, E_D: Combined fractions 17-20. CIPRO: Ciprofloxacin (Positive control), Acetone: Negative control, ND: Not determined. Values are means of duplicate reading. Means followed by different superscript letters in the table above are significantly different at P<0.0001.

Table 3 shows seven hexane fractions of *W.ugandensis* roots that were obtained and evaluated for anti-*Salmonella* activity by microdilution assay. The MIC values of these fractions ranged from 1.22 to 312.5 µg/ml. It is evident from these results that *W. ugandensis* fractions had activity against all the *Salmonella* strains tested.

3.3 Identification of major compounds in fractions of methanol extracts of *T.diversifolia* leaves by GC-MS

The seven methanol fractions of *T.diversifolia* leaves designated T_A, T_B, T_C, T_D, T_E, T_F and T_G were subjected to GC-MS for identification of major compounds. Figure 1, 2, 3 and 4 are GC-MS chromatograms from analysis of fraction T_A, T_B, T_D and T_E respectively.

Retention times of 10.927 and 13.694 minutes are indicative of presence of, 2, 4-dimethylhexanoic and 3, 4, 5-trimethyl-1-hexene in fraction T_A. Other retention times were not linked to any known compound by GC-MS and were not identified in the NIST library database and need further exploration of the fraction to reveal identity

(Figure 1)

Table 3. MIC (µg/ml) of fractions of hexane extracts of *W.ugandensis* root

	Clinical isolates of *Salmonella* strains			
Plant Fractions	***S.* ser. Typhimurium (ATCC 1408)**	***S.*ser.Typhi (ATCC 13347)**	***S.*ser.Typhi (ATCC 43579)**	***S.enterica* (ATCC 2162)**
H_A	1.22 ^{d}	1.22 ^{d}	156.25^{bc}	312.5^{a}
H_B	1.22 ^{d}	1.22 ^{d}	78.13 ^{cd}	312.5 ^{a}
H_C	1.22 ^{d}	1.22 ^{d}	1.22 ^{d}	156.25^{bc}
H_D	39.06 ^{d}	1.22 ^{d}	1.22 ^{d}	1.22 ^{d}
H_E	19.53 ^{d}	312.5	156.25 ^{bc}	312.5 ^{a}
H_F	78.13^{cd}	1.22 ^{d}	1.22 ^{d}	9.77 ^{d}
H_G	78.13 ^{cd}	19.53 ^{d}	4.88 ^{d}	1.22 ^{d}
CIPRO	19.53 ^{d}	1.22 ^{d}	9.77 ^{d}	1.22 ^{d}
Acetone	ND	ND	ND	ND

H: Hexane *W.ugandensis* root, H_A: Combined fractions1-16, H_B: Combined fractions 17-23, H_C: Combined fractions 24-39, H_D: Combined fractions 40-58, H_E: Combined fractions 59-69, H_F: Combined fractions 70-89 and H_G: Combined fractions 90-1000, CIPRO: Ciprofloxacin (Positive control) Acetone: Negative control, ND: Not determined. Values are means of duplicate reading. Means followed by different superscript letters in the table above are significantly different at P<0.0001.

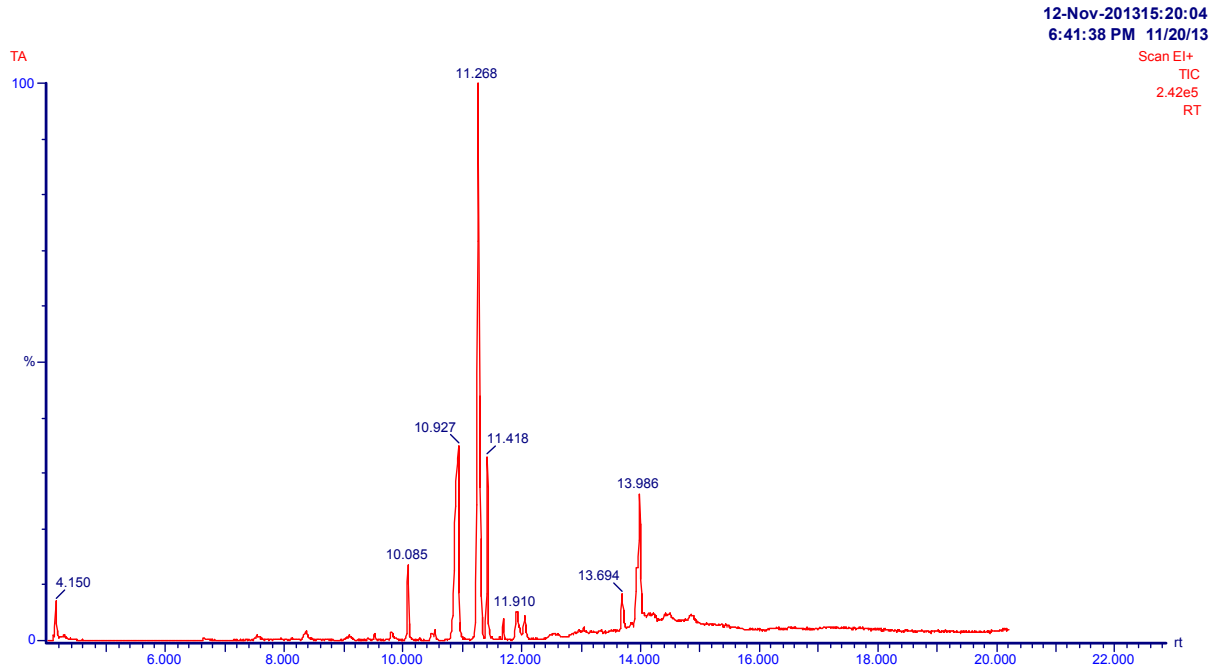

Figure 1. Chromatogram of T_A of methanol extract of *T.diversifolia*

In fraction T_B, peaks with retention times 10.927 and 11.994 were indicative of presence of n-hexadecanoic acid (palmitic acid) and 9, 12-octadecadienoic acid (Z, Z) compounds respectively. Other peaks in Figure 2 were not linked to any known compounds.

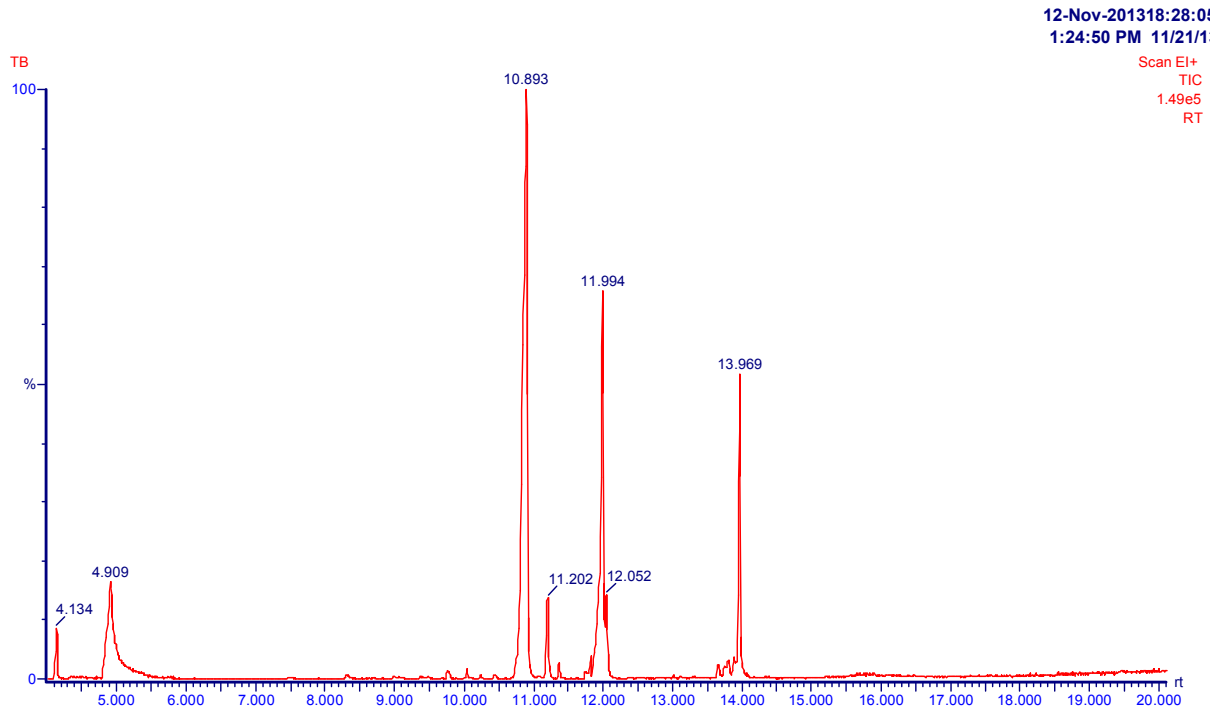

Figure 2: Chromatogram of T_B of methanol extract of *T.diversifolia*

In fraction T_D, peaks with retention times 5.344 and 9.376 were indicative of the presence 1, 2, 3-propanetriolmonoacetate and O-(2-methylpropyl) hydroxylamine compounds respectively (Figure 3).

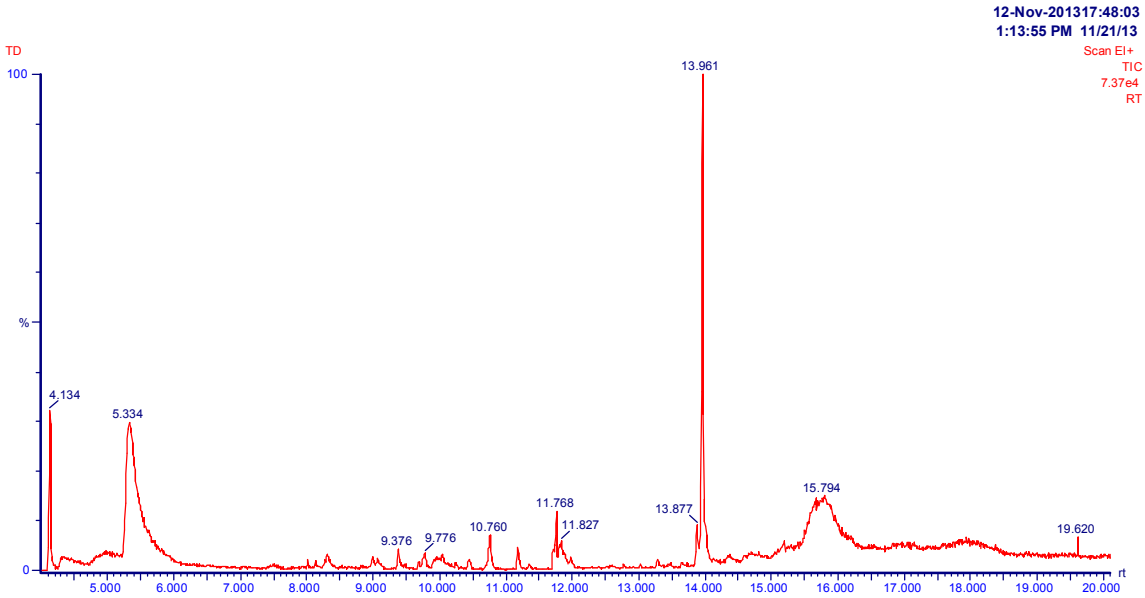

Figure 3. Chromatogram of T_D of methanol extract of *T.diversifolia*

In fraction T_E , peaks with retention times of 4.684, 9.318 and 15.086 are indicative of presence of, 1, 4:3,6-dianhydro – D- sorbitol, isorsorbide; E-2-Tetradecen-1-ol and Crotonic acid (o-formylphenylester compounds respectively (figure 4).

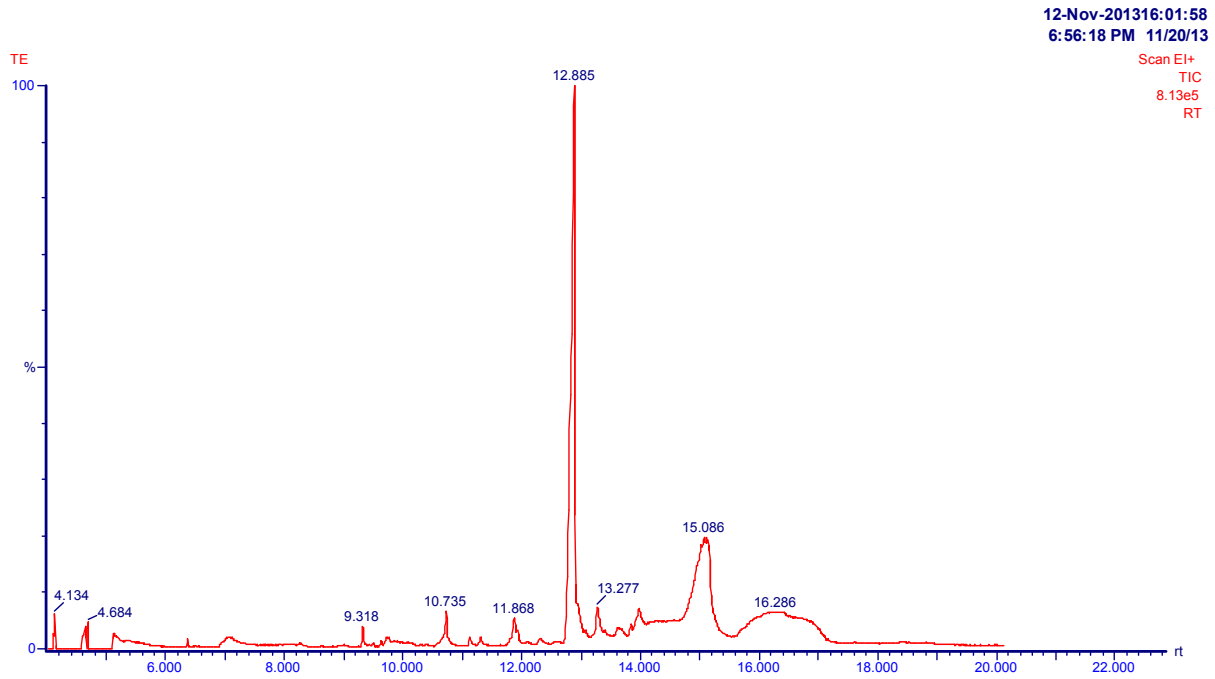

Figure 4. Chromatogram of T_E of methanol extract of *T.diversifolia*

Fraction T_F and T_G were not identified in the NIST library database and need further exploration of the fractions to reveal identity.

Table 4 illustrates the GC-Ms Retention Time (RT), molecular formula, and molecular weight of individual compounds of methanol fractions of *T. diversifolia* leaf.

Table 4. Components identified in methanol fraction of leaf of *T. diversifolia* by GC-MS analysis

Fraction	Peak	$T_{R(s)}$	M_F	M_W	Name of compound
T_A	1	10.927	$C_8H_{16}O_2$	144	2,4-dimethylhexanoic acid
	2	13.694	C_9H_{18}	126	3,4,5-trimethyl-1-hexene
T_B	1	10.893	$C_{16}H_{32}O_2$	256	n-hexadecanoic acid (palmitic acid)
	2	11.994	$C_{18}H_{32}O_2$	280	9,12-octadecadienoic acid(Z,Z)
T_C	---	---	---	---	Not determined
T_D	1	5.334	$C_5H_{10}O_4$	134	1,2,3-propanetriolmonoacetate
	2	9.376	$C_4H_{11}NO$		O-(2-methylpropyl)hydroxylamine
T_E	1	4.684	$C_6H_{10}O_4$	146	1,4:3,6-dianhydro – D- sorbitol, isorsorbide
	2	9.318	$C_{14}H_{28}O$	212	E-2-Tetradecen-1-ol,Tetradecanal
	3	15.086	$C_{11}H_{10}O_3$	190	Crotonic acid (o-formylphenylester)
T_F	---	---	---	---	Not determined
T_G	---	---	---	---	Not determined

$T_{R(s)}$: Retention time (seconds), M_F: Molecular formula and M_W: Molecular weight (grams)

3.4 Identification of major compounds in ethyl acetate extracts of *W.ugandensis* stem bark by GC-MS

The four ethyl acetate fractions of *W.ugandensis* stem bark designated E_A, E_B, E_C and E_D were subjected to GC-MS for identification of major compounds. Table 5 shows the active principles with their retention time (RT), molecular formula and molecular weight (MW) of the identified compounds in fractions E_A and E_D. Fractions E_B and E_C were not linked to any known compounds in the NIST library database and need further exploration to reveal identity.

Table 5. Components identified in Ethyl acetate fraction of stem bark of *W.ugandensis* by GC-MS analysis

Fraction	Peak	$T_{R(s)}$	M_F	MW	Name of compound
E_A	1	6.359	$C_{13}H_{28}$	184	6-ethyl-2-methyldecane
	2	7.543	$C_{15}H_{24}$	204	Bicyclo[7.2.0]undec-4-ene,4,11,11-trimethyl-8-methylene-
	3	7.801	$C_{15}H_{24}$	204	1,4,8-cycloundecatriene-2,6,6-carophylene
	4	9.235	$C_{15}H_{26}O$	222	1,6,10-Dodecantrien-3-ol, 3,7,11-trimethyl-(E)-
	5	10.393	Unknown	Unknown	3-ethenyl-3-methyl-2,1-(1-methylethenyl)-6-(1-methylethyl)cyclohexanol
	6	14.011	$C_{22}H_{42}O_4$	370	Hexanedioic acid, bis(2-ethylhexyl)ester
E_D	1	10.843	$C_{16}H_{32}O_2$	256	Hexadecanoic acid/Palmitic acid
	2	11.910	$C_{10}H_{10}O_4$	252	E-15-heptadecanal

$T_{R(s)}$: Retention time (seconds), M_F: Molecular formula and M_W: Molecular weight (grams)

3.5 Identification of major compounds in hexane extracts of *W.ugandensis* roots by GC-MS

The seven hexane fractions of *W.ugandensis* root designated H_A, H_B, H_C, H_D, H_E, H_F and H_G were subjected to GC-MS analysis. Table 7 shows retention time, molecular weight and molecular formula of known compounds identified in fraction H_A, H_B and H_F. Fractions; H_C, H_D, H_E and H_G were not identified in the NIST library database and need further exploration of these fractions to reveal identity.

Table 6. Components identified in hexane fraction of root of *W.ugandensis* by GC-MS analysis

Fraction	Peak	$T_{R(s)}$	M_F	M_W	Name of compound
H_A	1	5.884	$C_{13}H_{28}$	184	6-ethyl-2-methyldecane
	2	6.384	$C_{17}H_{28}O_2$	264	Nerolidyl acetate
	3	6.993	$C_{15}H_{24}$	204	Beta Sesquiphellandrene
	4	9.776	$C_{18}H_{36}O_4$	316	9,10-dihydroxyloctadecanoic acid
	5	11.218	$C_{17}H_{32}O$	252	E-15-heptadecenal
	6	13.894	$C_{24}H_{38}O_4$	390	1,2-benzenedicarboxylic acid, diiisooctyl,
	7	19.410	$C_{13}H_{18}O_2$	206	2-cylohexane-1-one,2,4,4-trimethyl-3-(3-oxo-butenyl)-
H_B	1	4.867	$C_{21}H_{44}$	296	Heptadecane,2,6,10,14-tetramethyl-
	2	7.734	$C_{15}H_{26}O$	242	(S,3E,7E)-α,α,4,8-Tetramethyl-3,7-cyclodecadiene-1-methanol
	3	9.151	$C_{15}H_{26}O$	242	(2,5,5,8a-Tetramethyl-1,4,4a,5,6,8,8a-octahydro-1-napthlenyl)methanol
	4	11.594	$C_{15}H_{24}$	234	Spiro[5,5]undec-2-ene,3,7,7trimethyl-11 methylene(chamigrene)
	5	11.836	$C_{20}H_{32}O_8$	456	Pentacyclo[9.1.0.0(2,4).0(5,7).0(8,10)]dodecane
	6	12.053	$C_{15}H_{22}O_2$	384	(5aS,9aS,9bR)-6,6,9a-Trimethyl-5,5a,6,7,8,9,9a,9b-octahydronaphtho[1,2-c]furan-1(3H)-one (drimenol)
H_F	1	11.677	$C_{17}H_{32}O$	252	E-15-Heptadecenal
	2	12.994	$C_{15}H_{26}O_3$	284	1,1,4,6-Tetramethyldecahydro-1H-cyclopropa[e]azuelene-4,5,6-triol
	3	13.902	$C_{24}H_{38}O_4$	390	1,2-benzenedicarboxylic acid,diisooctyl ester

$T_{R(s)}$: Retention time (seconds), M_F: Molecular formula and M_W: Molecular weight (grams)

4. Discussion

Methanol extract of *T.diversifolia* leaf, ethyl acetate extract and hexane extract of stem bark and root of *W.ugandensis* respectively were fractionated on silica column and fractions obtained were analyzed by silica TLC. The TLC results indicated that seven methanol fractions of *T.diversifolia* leaf designated T_A, T_B, T_C, T_D, T_E, T_F and T_G, four ethyl acetate fractions of *W.ugandensis* stem bark designated E_A, E_B, E_C and E_D and seven hexane fractions of *W.ugandensis* root designated H_A, H_B, H_C, H_D, H_E, H_F and H_G were collected. All fractions were evaluated for anti-*Salmonella* activity by microdilution assay.

The MICs values of the seven methanol fractions of *T.diversifolia*; T_A, T_B, T_C, T_D, T_E, T_F and T_G were in the range of 1.22-312.5µgml^{-1} against the 4 clinical isolates of *Salmonella* strains. The MIC values of T_A were 9.77, 2.44, 4.88 and 4.88 µg/ml against *S.* ser. Typhimurium (ATCC 1408), *S.*ser.Typhi (ATCC 13347), *S.*ser.Typhi (ATCC 43579), *S.enterica* (ATCC 2162) respectively. The MIC value as low as 2.44 µg/ml of a semi purified fraction against the clinical isolates of *Salmonella* strains is suggestive of good anti-*Salmonella* activity of the compounds of T_A. The MIC values of T_B were in the range of 1.22-312.5µg/ml against the four strains tested. The lowest MIC value noted for this fraction was 1.22 µg/ml against *S.*ser.Typhi (ATCC 13347) and *S.enterica* (ATCC 2162) whereas *S.* ser. Typhimurium (ATCC 1408) was the least sensitive with MIC value of 312.5 µg/ml. The MIC values for fraction T_C, T_D, T_E, T_F and T_G were in the range of 1.22-312.5 µg/ml. It was noted in our study that clinical *Salmonella* strains were sensitive to all methanol fractions of *T. diversifolia* at different MIC values. This compared well with ciprofloxacin broad spectrum antibiotics, which gave MIC values of 1.22 to 19.53 µg/ml and there was no significance difference in the activity observed (P<0.0001). The observed anti-*Salmonella* activity of *T. diversifolia* fractions agrees with the finding of Obafemi *et al*., (2006), on broad spectrum antimicrobial activity on germacranolide type sesquiterpene lactone from *Tithonia diversifolia* leaf extract (MICs = 15.6 – 62.5mg/ml for most strains of bacteria tested). Meffo *et al*., (2006), has also reported that tithoniaquinone A isolated from leaf of *T. diversifolia* showed strong antibacterial activity against the Gram-positive bacterium *Bacillus megaterium* and antifungal activity against *Microbotryumviolaceum.*

The MIC values of the four ethyl acetate fractions of *W.ugandensis* stem bark designated E_A, E_B, E_C and E_D ranged from 1.22 to 312.5 µg/ml. In the present study, fraction E_C and E_D showed remarkable anti-*Salmonella* activity of 1.22 µg/ml against *S.* ser. Typhimurium (ATCC 1408), *S.*ser.Typhi (ATCC 13347) and *S.enterica* (ATCC 2162). In addition, fraction E_C and E_D had MIC values of 19.53 and 9.77 µg/ml respectively against *S.*ser.Typhi (ATCC 43579). Similarly, fraction E_A and E_B also exhibited appreciable amount of anti-*Salmonella* activity. The fractions of ethyl acetate (stem bark) showed anti-*Salmonella* activity against all strains tested. Anti-*Salmonella* activity of *W.ugandensis* fractions compared well with standard drug, ciprofloxacin broad spectrum antibiotics and there was no significance difference in the observed activities (P<0.0001). The observed anti-*Salmonella* activity of *W.ugandensis* is however supported by Yibeltal *et al*., (2013) who demonstrated activity of crude and semi-purified fractions of *W.ugandensis* against *Shigella boydii* and *Staphylococcous aureus*. Studies carried out by Olila *et al*., (2001) on aqueous extracts of *W.ugandensis* stem bark showed activity against both *Escherischia coli* and *Staphylococcous aureus* in agar well assays but not in disc diffusion assay. The anti-*Salmonella* activity of *W.ugandensis* observed in our present study could be attributed to several secondary metabolites, among them steroids, terpenoids and glycosides. This was supported by Ogoti *et al*., (2015), who reported on the activity of secondary metabolites found in the crude extracts of *W.ugandensis* stem bark.

The MIC values of seven hexane fractions of *W.ugandensis* root were determined. Fractions designated H_A, H_B, H_C, H_D, H_E, H_F and H_G exhibited remarkable anti-*Salmonella* activity in the range of 1.22 to 312.5µgml^{-1} against the 4 clinical isolates of *Salmonella* strains. Fraction H_D showed higher anti-*Salmonella* activity against three of the four clinically isolated *Salmonella* strain. Fraction H_D had MIC value of 1.22 µg/ml against *S.*ser.Typhi (ATCC 13347), *S.* ser.Typhi (ATCC 43579), *S. enterica* (ATCC 2162). It also exhibited MIC value of 39.06µg/ml against *S.*ser.Typhimurium ATCC 1408. Fraction H_E demonstrated the least anti-*Salmonella* activity against three out of the four strains tested. The fraction had MIC values of 312.5, 156.25 and 312.5 µgml^{-1} against *S.*ser.Typhi (ATCC 13347), *S.*ser.Typhi (ATCC 43579), *S.enterica* (ATCC 2162) respectively. However, it showed MIC value of 19.53 µg/ml against *S.*ser.Typhimurium (ATCC 1408). The hexane fractions of *W.ugandensis* root showed appreciable amount of anti-*Salmonella* activity against all strains tested. Anti-*Salmonella* activity of *W.ugandensis* (root) fractions compared well with the activity of ciprofloxacin (1.22-19.53 µg/ml). The present study has demonstrated lower or equal MIC values for *W.ugandensis* fractions (root) against *Salmonella* strains tested that are comparable to those of ciprofloxacin broad spectrum antibiotics. Therefore, anti-*Salmonella* activity for the fractions and that of ciprofloxacin had no significant difference (p<0.0001).

Our present study has demonstrated lower MIC values for *W.ugandensis* fractions (root) against *Salmonella* strains tested than what Yibeltal, *et al*., (2013), reported. According to their report, MIC values of semi-purified fraction of petroleum ether extract of *W.ugandensis* (heartwood) against both *S.boydii* and *S.aureus* was 500 µg/ml (0.5mg/ml) and 1000 µg/ml (1mg/ml) against *E.coli*. In addition, *Candida albicans* had MIC value of 1000 µgml^{-1} for semi-purified fraction of petroleum ether extracts of *W.ugandensis* both the leaf and the heartwood

(Yibeltal, *et al*., 2013). Results of growth inhibitory activity exhibited on the clinical isolates of *Salmonella* strains by hexane fractions of *W.ugandensis* root indicated the plant contained anti-*Salmonella* agents which supported its use in the local treatment of typhoid fever.Therefore the observed anti-*Salmonella* activity of fractions of *W.ugandensis* root in our present study could be attributed to several secondary metabolites, among them steroids, terpenoids and glycosides. These phytochemical compounds have been demonstrated to have anti-*Salmonella* activity by Ogoti *et al*., (2015).

A total of eighteen semi purified fractions from methanol extracts of *T.diversifolia* leaf, ethyl acetate stem bark and hexane root extracts of *W.ugandensis* were analyzed by GC-MS technique to identify the major compounds. Our study showed the presence of alkenes, fatty acids and short chain unsaturated carboxylic acid in the active methanol, ethyl acetate and hexane fractions. Nine important compounds were identified in methanol fractions based on the database in the NIST library. For instance, 9, 12-octadecadienoic acid (Z, Z) and n-hexadecanoic acid identified by GC-MS analysis are fatty acids whereas 3, 4, 5-trimethyl-1-hexene detected was alkenes. Crotonic acid identify in fraction T_E is a short chain unsaturated carboxylic acid, among others. Meanwhile, eight important compounds were detected in the ethyl acetate fractions of *W.ugandensis* stem barks. Some of them include; hexanedioic acid, bis (2-ethylhexyl) ester, Hexadecanoic acid and E-15-heptadecanal. Likewise sixteen important compounds were also successfully identified in the hexane fractions of *W.ugandensis* root. E-15-heptadecenal, 1, 2-benzenedicarboxylic acid and 6-ethyl-2-methyldecane were detected among others.

Majority of the phytoconstituens identified in methanol, ethyl acetate and hexane fractions are attributed with various biological activities. For example, hexadecanoic acid is a very common saturated fatty acid, known anti-inflammtory phytoconstituent as it is a phospholipase inhibitor (Aparna *et al*., 2012) and it's also known for its antibacterial activity (Manilal *et al*., 2009). The n-hexadecanoic acid is also a known fatty acid that possesses antioxidant, hypocholesterolemic, nematicide, pesticide and antiandrogenic activity (Duke, 2007). Likewise 9,12-octadecadienoic acid (Z,Z), is otherwise called as omega 6 fatty acids which are a family of pro-inflammatory, anti-inflammatory polyunsaturated fatty acid and antimicrobial agents (Marimuthu *et al*., 2014). The 1, 2-benzenedicarboxylic acid and beta-sesquiphellandrene have been shown to have antimicrobial property (Duke *et al*., 2007, Vukovic *et al*., 2007). E-15-Heptadecenal, an aldehyde was identified in both hexane and ethyl acetate extract of *W.ugandensis*, has been reported for antibacterial activity (Vinay *et al*., 2011).

5. Conclusion

This study confirms the presence of therapeutically potent anti-*Salmonella* compounds in the methanol fractions of *T.diversifolia* leaf, ethyl acetate and hexane fractions of stem bark and root of *W.ugandensis* respectively that could lead to development of antibiotics against typhoid fever. Further work is in progress to determine the effect of active compounds on dihydrofolate reductase.

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