**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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**Keywords:** *T. diversifolia, W. ugandesis*, Anti-*Salmonella* activity, Microdilution assay, Silica gel column chromatography, GC-MS

**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 37⁰C. 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 TA, TB, TC, TD, TE, TF and TG were obtained after silica TLC analysis. Likewise four ethyl acetate fractions of *W.ugandensis* bark designated EA, EB, EC and ED were determined by silica TLC analysis. Meanwhile, seven hexane fractions of *W.ugandensis* root designated HA, HB, HC, HD, HE, HF and HG 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 (TA, TB, TC, TD, TE, TF, TG) 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**

|  |  |
| --- | --- |
| **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)** |
| TA | 9.77d | 2.44 d | 4.88 d | 4.88 d |
| TB | 312.5a | 1.22 d | 19.53 d | 1.22 d |
| TC | 78.13cd | 1.22 d | 78.13 cd | 1.22 d |
| TD | 39.06 d | 1.22 d | 19.53 d | 39.06 d |
| TE | 39.06 d | 1.22 d | 19.53 d | 39.06 d |
| TF | 39.06 d | 1.22 d | 78.13 cd | 312.5 a |
| TG | 19.53 d | 4.88 d | 78.13 cd | 2.44 d |
| CIPRO | 19.53 d | 1.22 d | 9.77d | 1.22 d |
| Acetone | ND | ND | ND | ND |

T:*T.diversifolia* leaf, TA: Combined fractions1-10, TB: Combined fractions 11-17, TC: Combined fractions 18-24, TD: Combined fractions 25-32, TE: Combined fractions 33-39, TF: Combined fractions 40-44 and TG: 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)** |
| EA | 156.25 bc | 19.53 **d** | 156.25bc | 156.25bc |
| EB | 9.77d | 2.44 d | 312.5a | 9.77 d |
| EC | 1.22 d | 1.22 d | 19.53 d | 1.22 d |
| ED | 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, EA: Combined fractions 1-6, EB: Combined fractions 7-11, EC: Combined fractions 12-16, ED: 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 TA, TB, TC, TD, TE, TF and TG were subjected to GC-MS for identification of major compounds. Figure 1, 2, 3 and 4 are GC-MS chromatograms from analysis of fraction TA, TB, TD and TE 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 TA. 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**

|  |  |
| --- | --- |
| **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)** |
| HA | 1.22 **d** | 1.22 **d** | 156.25bc | 312.5a |
| HB | 1.22 **d** | 1.22 **d** | 78.13 cd | 312.5 a |
| HC | 1.22 **d** | 1.22 **d** | 1.22 **d** | 156.25bc |
| HD | 39.06 **d** | 1.22 **d** | 1.22 **d** | 1.22 **d** |
| HE | 19.53 **d** | 312.5 | 156.25 bc | 312.5 a |
| HF | 78.13cd | 1.22 **d** | 1.22 **d** | 9.77 **d** |
| HG | 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, HA: Combined fractions1-16, HB: Combined fractions 17-23, HC: Combined fractions 24-39, HD: Combined fractions 40-58, HE: Combined fractions 59-69, HF: Combined fractions 70-89 and HG: 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 TA of methanol extract of *T.diversifolia*

In fraction TB, 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 TB of methanol extract of *T.diversifolia*

In fraction TD, 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 TD of methanol extract of *T.diversifolia*

In fraction TE , 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 TE of methanol extract of *T.diversifolia*

Fraction TF and TG 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** | **TR(s)** | **MF** | **MW** | **Name of compound** |
| TA | 1 | 10.927 | C8H16O2 | 144 | 2,4-dimethylhexanoic acid |
|  | 2 | 13.694 | C9H18 | 126 | 3,4,5-trimethyl-1-hexene |
| TB | 1 | 10.893 | C16H32O2 | 256 | n-hexadecanoic acid (palmitic acid) |
|  | 2 | 11.994 | C18H32O2 | 280 | 9,12-octadecadienoic acid(Z,Z) |
| TC | --- | --- | --- | --- | Not determined |
| TD | 1 | 5.334 | C5H10O4 | 134 | 1,2,3-propanetriolmonoacetate |
|  | 2 | 9.376 | C4H11NO |  | O-(2-methylpropyl)hydroxylamine |
| TE | 1 | 4.684 | C6H10O4 | 146 | 1,4:3,6-dianhydro – D- sorbitol, isorsorbide |
|  | 2 | 9.318 | C14H28O | 212 | E-2-Tetradecen-1-ol,Tetradecanal |
|  | 3 | 15.086 | C11H10O3 | 190 | Crotonic acid (o-formylphenylester) |
| TF | --- | --- | --- | --- | Not determined |
| TG | --- | --- | --- | --- | Not determined |

TR(s): Retention time (seconds), MF: Molecular formula and MW: 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 EA, EB, EC and ED 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 EA and ED. Fractions EB and EC 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** | **TR(s)** | **MF** | **MW** | **Name of compound** |
| EA | 1 | 6.359 | C13H28 | 184 | 6-ethyl-2-methyldecane |
|  | 2 | 7.543 | C15H24 | 204 | Bicyclo[7.2.0]undec-4-ene,4,11,11-trimethyl-8-methylene- |
|  | 3 | 7.801 | C15H24 | 204 | 1,4,8-cycloundecatriene-2,6,6-carophylene |
|  | 4 | 9.235 | C15H26O | 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 | C22H42O4 | 370 | Hexanedioic acid, bis(2-ethylhexyl)ester |
| ED | 1 | 10.843 | C16H32O2 | 256 | Hexadecanoic acid/Palmitic acid |
|  | 2 | 11.910 | C10H10O4 | 252 | E-15-heptadecanal |

TR(s): Retention time (seconds), MF: Molecular formula and MW: 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 HA, HB, HC, HD, HE, HF and HG were subjected to GC-MS analysis. Table 7 shows retention time, molecular weight and molecular formula of known compounds identified in fraction HA, HB and HF. Fractions; HC, HD, HE and HG 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** | **TR(s)** | **MF** | **MW** | **Name of compound** |
| HA | 1 | 5.884 | C13H28 | 184 | 6-ethyl-2-methyldecane |
|  | 2 | 6.384 | C17H28O2 | 264 | Nerolidyl acetate |
|  | 3 | 6.993 | C15H24 | 204 | Beta Sesquiphellandrene |
|  | 4 | 9.776 | C18H36O4 | 316 | 9,10-dihydroxyloctadecanoic acid |
|  | 5 | 11.218 | C17H32O | 252 | E-15-heptadecenal |
|  | 6 | 13.894 | C24H38O4 | 390 | 1,2-benzenedicarboxylic acid, diiisooctyl, |
|  | 7 | 19.410 | C13H18O2 | 206 | 2-cylohexane-1-one,2,4,4-trimethyl-3-(3-oxo-butenyl)- |
| HB | 1 | 4.867 | C21H44 | 296 | Heptadecane,2,6,10,14-tetramethyl- |
|  | 2 | 7.734 | C15H26O | 242 | (S,3E,7E)-α,α,4,8-Tetramethyl-3,7-cyclodecadiene-1-methanol |
|  | 3 | 9.151 | C15H26O | 242 | (2,5,5,8a-Tetramethyl-1,4,4a,5,6,8,8a-octahydro-1-napthlenyl)methanol |
|  | 4 | 11.594 | C15H24 | 234 | Spiro[5,5]undec-2-ene,3,7,7trimethyl-11 methylene(chamigrene) |
|  | 5 | 11.836 | C20H32O8 | 456 | Pentacyclo[9.1.0.0(2,4).0(5,7).0(8,10)]dodecane |
|  | 6 | 12.053 | C15H22O2 | 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) |
| HF | 1 | 11.677 | C17H32O | 252 | E-15-Heptadecenal |
|  | 2 | 12.994 | C15H26O3 | 284 | 1,1,4,6-Tetramethyldecahydro-1H-cyclopropa[e]azuelene-4,5,6-triol |
|  | 3 | 13.902 | C24H38O4 | 390 | 1,2-benzenedicarboxylic acid,diisooctyl ester |
| TR(s): Retention time (seconds), MF: Molecular formula and MW: 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 TA, TB, TC, TD, TE, TF and TG; four ethyl acetate fractions of *W.ugandensis* stem bark designated EA, EB, EC and ED and seven hexane fractions of *W.ugandensis* root designatedHA, HB, HC, HD, HE, HF and HG were collected. All fractions were evaluated for anti-*Salmonella* activity by microdilution assay.

The MICs values of the seven methanol fractions of *T.diversifolia*; TA, TB, TC, TD, TE, TF and TGwere in the range of 1.22-312.5µgml-1 against the 4 clinical isolates of *Salmonella* strains. The MIC values of TA 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/mlof a semi purified fraction against the clinical isolates of *Salmonella* strains is suggestive of good anti-*Salmonella* activity of the compounds of TA.The MIC values of TB 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 TC, TD, TE, TF and TG 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 EA, EB, EC and ED ranged from 1.22 to 312.5 µg/ml. In the present study, fraction EC and ED 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 EC and ED had MIC values of 19.53 and 9.77 µg/ml respectively against *S.*ser.Typhi (ATCC 43579). Similarly, fraction EA and EB 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 HA, HB, HC, HD, HE, HF and HG exhibited remarkable anti-*Salmonella* activity in the range of 1.22 to 312.5µgml-1 against the 4 clinical isolates of *Salmonella* strains. Fraction HD showed higher anti-*Salmonella* activity against three of the four clinically isolated *Salmonella* strain. Fraction HD 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 HE 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/mlagainst *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 offractions 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 TE is a short chain unsaturated carboxylic acid, among others.Meanwhile, eight important compounds were detected in theethyl 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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