# *In Vitro* Anti-salmonella Activity of Extracts from Selected Kenyan Medicinal Plants

**[1]P.Ogoti, [1]E.Magiri, [1]G.Magoma, [1]D.Kariuki, [2]C.Bii, [2]S.Kariuki**
[1]Department of Biochemistry, Jomo Kenyatta University of Agriculture and Technology,
P.o Box 62000-00200, Nairobi.
[2]Centre of Microbiology Research (CMR) KEMRI,
P.o Box 54840-00200.

Correspondence should be addressed to P. Ogoti,ogotim2002@yahoo.co.uk, +254700354243, +254728957828

## Abstract

The aim of this study was to determine *in vitro* anti-salmonella activity of n-hexane, ethyl-acetate, methanol and water extracts of 5 selected Kenyan medicinal plants against 5 *Salmonella typhi* strains and *Salmonella typhimurium* which were provided by the Centre of Microbiology Research-Kenya Medical Research Institute (CMR-KEMRI). The 5 plants namely *Warburgia ugandesis*, *Carissa edulis*, *Tithornia diversifolia, Croton megalocarpus* and *Launae cornuta* that are used traditionally in treatment of typhoid fever and other bacterial diseases were screened. The extracts from these plants were assayed for *in vitro* anti-salmonella activity using the disc diffusion and microdilution techniques to determine the zones of inhibition and Minimum Inhibitory Concentration (MIC).The results from the present study have shown that out of 36 extracts investigated; only eleven extracts from *W. ugendensis, T. diversifolia* and *C.megalocarpus* showed activity against S*almonella* strains at 1000mg/ml. The zone of inhibition range from 8mm to 18.5±0mm while 25 plant extracts that were not sensitive showed zone of inhibition of ≤6mm. MIC of the extracts was in the range of <0.031-15.63 mg/ml. The three plants with anti-salmonella activity can therefore be used to source antibiotic substances for drug development that can be used in the control of typhoid fever. The study therefore provides the scientific basis for its traditional application as a local health remedy.

**Keywords:** Anti-salmonella activity, medicinal plant extracts, Minimum Inhibitory Concentration, disc diffusion, Salmonella organisms

## 1.0 Introduction

*Salmonella enteric*, which are Gram-negative bacterial pathogens capable of infecting humans and animals, cause significant morbidity and mortality worldwide (Fink and Cookson, 2007) *S.enterica* serovar *typhimurium* is a clinically important intracellular bacterial pathogens that cause food poisoning and gastroenteritis in millions of people worldwide each year (Grassl, *et al*., 2008). The Centre for Disease Control (CDC) estimates that there are nearly 1.4 million food-borne *Salmonella* infections annually in USA (Mead, *et al*., 1999). This bacterium infects the intestinal tract and causes systemic infection of various organs such as the liver and spleen (Mead, *et al*., 1999).

Fluoroquinolones and tetracyclines are the antibiotics most commonly used to treat *Salmonella*, and until recently most strains were susceptible to these drugs. However, a high incidence of *Salmonella* strains resistant to commonly prescribed antibiotics has been reported in Korea and other countries (Choi, *et al*., 2005, and Stevenson, *et al*., 2007) and the increased appearance of antibiotics resistant strains of *Salmonella* further exacerbates this problem (Bhan, *et al*., 2005). One major concern to public health has been the global dissemination of *S.typhimurium* Definitive Type 104, which is commonly resistant to five or more antimicrobial agents (Perron, *et al*., 2008). The rise in antibiotic-resistant pathogens has led to the development of new

therapeutic agents that are effective against these bacteria. Currently, there has been considerable interest in the use of plant materials as alternative method to control pathogenic microorganisms (Aqil, *et al*., 2005) and many compounds of plant products have been shown to be specifically targeted against resistant pathogenic bacteria (Nostrol, *et al*., 2006). According to WHO more than 80% of world's population relies on traditional medicine for their primary healthcare, majority of them use plants or their active principles (*Gupta, et al*., 2005). Many plants are used in Africa continent for treatment of different diseases such as fever, dysentry, cholera, diarrhoea etc and others which are typical disease of a tropical country (Ayogu and Amadi, 2009; Ajaji and Akintola, 2010).Traditional medical practitioners in Kenya use herbal preparations to treat microbial infections such as typhoid and paratyphoid infections and they claimed that the primary benefit of using plant-derived medicines is that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments. Currently, attention has been directed towards medicinal plant research to substantiate the claims of the cure made by traditional healers thus providing scientific basis for their efficacy. These medicinal plants; *Warburgia ugandensis* (root and bark), *Carissa endulis* (root and bark), *Launae cornuta* (root and bark), *Tithornia diversifolia* (Leaf and flower), and *Croton megalocarpus* (bark) have been claimed by traditional medical practitioners in Kenya to be effective when used for treatment of fevers, particularly typhoid fever. However, no detailed reports on anti-typhoid activity of these plants exist in literature in Kenya, therefore; the present study investigates the anti-typhoid activities of extracts of these five medicinal plants.

## 2.0 Methods

### 2.1 Salmonella strains and Culture medium

*S.typhi* ATCC 13347, *S.typhi* ATCC 43579, *S.enterica* ATCC 2162, *S.typhi* ATCC 014112, *S.typhi* ATCC 01617 and *S.typhi*murium ATCC 1408, which were provided by the Centre of Microbiology Research-Kenya Medical Research Institute (CMR-KEMRI) were used in this study. The *Salmonella* stains were suspended in Muller Hinton Broth (MHB, Difco, USA) and then incubated at 37 $^{0}$ for 24 h. Muller Hinton media was used for the disc diffusion method.

### 2.2. Extraction of plant materials

#### 2.2.1 Sequential extraction of active compounds from plant materials by organic solvents

Five plants selected for this study were collected from various locations in Nyamira County as indicated in Table 1. The plants were then authenticated from Jomo Kenyatta University of Agriculture and Technology, Botany Department. The plant materials were taken to the laboratory where they were carefully washed under running tap water and left to drain off. Then, the plant materials were air-dried in the laboratory for three weeks and thereafter ground into a powder by a mechanical grinder. Approximately 500g of each of the powdered plant materials were soaked separately with 1500ml of hexane into conical flasks with a rubber cork. The contents were kept for 3 days then filtered through sterile filter paper into clean conical flasks. The filtrate were transferred into sample holder of rotary vacuum evaporator and the hexane solvent evaporated at its boiling temp of 38.5-42$^{o}$C. The residues obtained from hexane extraction were re-soaked in 1500 ml of ethyl acetate in conical flask with a rubber cork. The contents were kept for 5 days away from direct sunlight, undisturbed, then, were filtered through sterile filter paper into a clean conical flask. The filtrate was transferred into sample holder of rotary vacuum evaporator and the ethyl acetate solvent evaporated at its boiling temp of 38.5-

42ºC. And finally, the residues of ethyl acetate extraction were re-soaked in 1500 ml of methanol in a conical flask with a rubber cork for 36 hours away from direct sunlight, undisturbed; then, were filtered through sterile filter paper into a clean conical flask. The filtrate was transferred into sample holder of rotary vacuum evaporator where the methanol solvent was evaporated at its boiling temperature of 65ºC. The extracts obtained were stored in refrigerator at 4º C until required for use.

### 2.2.2 Aqueous extraction

Five hundred grams (500g) of plant material were weighed out and soaked separately in 1500ml of distilled water in a conical flask. The contents were warmed in a water bath for 2 hours at 60ºC, then left to stand at room temperature for 10 hours, undisturbed. They were subsequently filtered off with sterile filter paper (whatman No. 1) into a clean conical flask and the filtrate was freeze dried to powder, weighed and stored until required for use.

### 2.3. Determination of phytochemical constituents

The freshly prepared extracts were subjected to standard phytochemical analyses for tannins, alkaloids, terpenoids, flavanoids, glycosides, steroids and saponin as described by Jigna, *et al*., 2006.

### 2.4. Disc diffusion Method

Circular paper discs 6mm diameter were provided by KEMRI-CMR and then put in a petri dishes containing innoculated Muller Hinton media. Ten micro-liters of 1000mg/ml of plant extracts was transferred by pippette and spotted on each disc. Petri dishes inoculated with *Salmonella* strains and were kept for incubation for 24 h at 37 $^{0}$. The diameter of growth inhibition zones were measured using a ruler and compared to DMSO as negative control. Chloramphenicol and ciprofloxacin were used as positive control.

### 2.5. Microdilution Method

To determine the MIC values, the microdilution assay as described by Eloff (1998) was followed. Chlorampenicol and ciprofloxacin were used as a positive control; DMSO was used as negative control. Plant extracts were tested against *Salmonella* strains with varying concentration starting from 62.5mg/ml-0.0305mg/ml. Briefly, 100 µl of sterile distilled water was added to 96-well microtitre plates followed by the addition of 100 µl of 62.5mg/ml of the plant extracts and serially diluted two fold after which 100 µl of *Salmonella* strains were added to each microtitre plates in all the wells to give the final volume of 200 µl. The prepared microtitre plates were sealed to avoid drying and incubated overnight at 37$^{0}$C in 100% relative humidity. After overnight incubation, 50µl of 5mg/ml 2, 3, 5 Triphenyltetrazolium chloride dissolved in water was then added to all the microtitre plate wells and incubated for overnight again. Bacteria growth was indicated by the pink colour while the growth inhibition was indicated by less or lack of pink colour. The MIC values were then recorded as the lowest concentration at which a decrease in pink colour is apparent compared to the next dilution after 24h of incubation.

### 2.6 Statistical procedures

Anti-salmonella activity test were performed in triplicates. Average values with standard deviation (SD) are presented in the Table 3.

## 3.0 Results

**Table 1.Profile of the five medicinal plants used**

| Botanical name | Family name | Localities | Part of the plant used |
|---|---|---|---|
| *Warburgia ugandesis* | Conellaceae | Manga | Roots and barks |
| *Carissa edulis* | Apocynaceae | Kionyo | Roots and barks |
| *Launae cornuta* | Asteraceae | Motobo | Roots and leaves |
| *Tithornia diversifolia* | Solanaceae | Motobo | Flowers and leaves |
| *Croton megalocarpus* | Euphorbiaceae | Morako | Barks |

**Table 2. Anti-typhoid activity of hexane, ethyl acetate, methanol and aqueous extracts**

| Medicinal plants | Sensitivity (%) | Resistance (%) |
|---|---|---|
| **Hexane extract** | | |
| *Wurburgia udensisis*(Root) | 6(100) | 0(0) |
| *Wurburgia udensisis*(Bark) | 6(100) | 0(0) |
| *Carissa edulis*(Bark) | 0(0) | 6(100) |
| *Carissa edulis*(Bark | 0(0) | 6(100) |
| *Launae cornuta*(Root) | 0(0) | 6(100) |
| *Launae cornuta*(Leaf) | 0(0) | 6(100) |
| *Tithornia diversifolia*(Flower) | 6(100) | 0(0) |
| *Tithornia diversifolia*(Leaf) | 1(17) | 5(83) |
| *Croton megalocarpus*(Bark) | 0(0) | 6(100) |
| **Ethyl acetate extract** | | |
| *Wurburgia udensisis*(Root) | 6(100) | 0(0) |
| *Wurburgia udensisis*(Bark) | 6(100) | 0(0) |
| *Carissa edulis*(Bark) | 0(0) | 6(100) |
| *Carissa edulis*(Bark | 0(0) | 6(100) |
| *Launae cornuta*(Root) | 0(0) | 6(100) |
| *Launae cornuta*(Leaf) | 0(0) | 6(100) |
| *Tithornia diversifolia*(Flower) | 1(17) | 5(83) |
| *Tithornia diversifolia*(Leaf) | 6(100) | 0(0) |
| *Croton megalocarpus*(Bark) | 1(17) | 5(83) |
| **Methanol extract** | | |
| *Wurburgia udensisis*(Root) | 0(0) | 6(100) |
| *Wurburgia udensisis*(Bark) | 0(0) | 6(100) |
| *Carissa edulis*(Bark) | 0(0) | 6(100) |
| *Carissa edulis*(Bark | 0(0) | 6(100) |
| *Launae cornuta*(Root) | 0(0) | 6(100) |
| *Launae cornuta*(Leaf) | 0(0) | 6(100) |
| *Tithornia diversifolia*(Flower) | 0(0) | 6(100) |
| *Tithornia diversifolia*(Leaf) | 6(100) | 0(0) |
| *Croton megalocarpus*(Bark) | 1(17) | 5(83) |
| **Aqueous extract** | | |
| *Wurburgia udensisis*(Root) | 0(0) | 6(100) |
| *Wurburgia udensisis*(Bark) | 0(0) | 6(100) |
| *Carissa edulis*(Bark) | 0(0) | 6(100) |
| *Carissa edulis*(Bark | 0(0) | 6(100) |
| *Launae cornuta*(Root) | 0(0) | 6(100) |
| *Launae cornuta*(Leaf) | 0(0) | 6(100) |
| *Tithornia diversifolia*(Flower) | 0(0) | 6(100) |
| *Tithornia diversifolia*(Leaf) | 0(0) | 6(100) |
| *Croton megalocarpus*(Bark) | 0(0) | 6(100) |
| **Controls** | | |
| Ciprofloxacin/chloramphenicol | 6(100) | 0(0) |
| DMSO | 0(0) | 6(100) |

The hexane and ethyl acetate extracts of *W.ugandensis* (root and bark) and hexane extracts of *T.diversifolia* flower and ethyl acetate extracts of *T.diversifolia* leaf and methanol extracts of *T.diversifolia* leaf were very active inhibiting 6(100%) of tested organism, only one extracts of hexane, one extract of methanol and two extracts of ethyl acetate inhibited 1(17%) of organism tested while other two plants namely *C. edulis*

and *L. cornuta* had no activity against the organisms tested. Chloramphenicol and ciprofloxacin had activity against 6(100%) of the tested organisms while the negative control, DMSO had no activity against the tested organisms

## Table 3. Anti-salmonella activity of hexane, ethyl acetate and methanol extracts of selected medicinal plants

| | Diameter of inhibition zone (mm) | | | | | |
|---|---|---|---|---|---|---|
| **Plant extracts** | ***S.typhi* ATCC 13347** | ***S.typhi* ATCC 43579** | ***S.enterica* ATCC 2162** | ***S.typhi* ATCC 014112** | ***S.typhi* ATCC 01617** | ***S.typhi*murium ATCC 1408** |
| WURE | 8.67±0.58 | 6±0 | 6.67±0.58 | 6±0 | 6±0 | 6.67±1.15 |
| WURH | 14±1 | 8.33±0.58 | 6.67±1.15 | 8±0 | 7.67±0.58 | 10.67±4.62 |
| WUBE | 6±0 | 7±0 | 6.33±0.58 | 6±0 | 6.67±0.58 | 6±0 |
| CMBE | 7.33±0.58 | 6±0 | 6±0 | 6.33±0.58 | 6.33±0.58 | 6.67±1.15 |
| WUBH | 11±3.21 | 7.33±2.31 | 6.33±0.58 | 7.33±0.58 | 6.67±1.15 | 7.33±0.58 |
| TDLE | 10±0 | 10±0.58 | 7.33±0.58 | 7±0 | 7.33±0.58 | 7.33±2.31 |
| TDFH | 15.67±2.08 | 15.75±1.15 | 7.33±1.15 | 6±0 | 8.33±2.31 | 6±0 |
| TDLM | 11±1 | 11.5±58 | 7.33±4.0.58 | 8±1.73 | 6.67±0.58 | 11.67±0.58 |
| CMBM | 6.67±0.58 | 6.75±1 | 7±1 | 6±0 | 6.67±0.58 | 6.67±1.15 |
| TDLH | 17.67±2.08 | 17±0 | 6±0 | 6±0 | 6±0 | 6±0 |
| TDFE | 18±2 | 18.5±0 | 6±0 | 7±0 | 6±0 | 6.67±0.58 |
| DMSO | 6±0 | 6±0 | 6±0 | 6±0 | 6±0 | 6±0 |
| CHLO | 23.33±0.58 | 24±1.73 | 24.33±0.58 | 22.33±0.58 | 23±1.73 | 6.67±0.58 |
| CIPRO | 26±2 | 23.33±2.52 | 26±0 | 26±2 | 25±1 | 19.67±1.53 |

**Key**: IZ=Inhibition zone (in mm) includes the diameter of the disc, WURE=*Warburgia ugandensis* root extract of ethyl acetate, WURH= *Warburgia ugandensis* root extract of hexane, WUBE= *Warburgia ugandensis* bark extract of ethyl acetate, CMBE= *Croton megalocarpus* bark extract of ethyl acetate, WUBH= *Warburgia ugandensis* bark extract of hexane, DMSO=Dimethyl sulphur dioxide, CHLO=Chloramphenicol, CIPRO=Ciprofloxacin, Values are means of triplicate readings (Means±SD).

## Table 4. Minimum Inhibitory Concentration (mg/ml) of Hexane, ethyl acetate and methanol extracts

| | Salmonella organisms | | | |
|---|---|---|---|---|
| **Plant extracts** | ***S.typhi* ATCC 13347** | ***S.typhi* ATCC 43579** | ***S.enterica* ATCC 2162** | ***S.typhi*murium ATCC 1408** |
| WURE | 0.24 | <0.031 | 0.061 | 0.12 |
| WURH | <0.031 | 0.031 | 3.91 | <0.031 |
| WUBE | <0.031 | <0.031 | 0.061 | <0.031 |
| WUBH | <0.031 | <0.031 | 0.488 | <0.031 |
| TDLE | 0.24 | 0.061 | <0.031 | 0.98 |
| TDLH | 0.24 | 0.24 | 1.95 | 0.488 |
| TDLM | 0.24 | <0.031 | 0.98 | <0.031 |
| TDFH | 0.98 | 0.12 | 3.91 | 3.91 |
| TDFE | 0.98 | 15.63 | 0.12 | 3.91 |
| DMSO | ND | ND | ND | ND |
| CHLO | 0.022 | 0.029 | 0.024 | 0.030 |
| CIPRO | 0.02 | 0.015 | 0.018 | 0.025 |

Key: ND =Not defined

**Table 5. Phytochemical constituents of the selected medicinal plant extracts**

| Plant extracts | Alkaloids | Saponin | Tannins | Flavanoids | Steroids | Terpenoids | Glycosides |
|---|---|---|---|---|---|---|---|
| WURE | + | - | + | + | ++ | ++ | - |
| WURH | - | - | - | + | ++ | ++ | - |
| WUBE | + | - | + | + | + | + | ++ |
| WUBH | - | - | - | + | ++ | ++ | - |
| TDLE | + | - | ++ | + | - | - | + |
| TDLH | - | - | - | - | +++ | + | - |
| TDLM | + | ++ | ++ | + | - | - | - |
| TDFE | + | - | + | + | +++ | ++ | - |
| TDFH | - | - | - | - | +++ | + | - |

**Key:-=absent; +=present; ++=Moderate concentration; +++=High concentration**

Profile of the five medicinal plants used are presented in Table 1.Three out of five plants screened namely *W. ugendensis,T diversifolia* and *C.megalocarpus* showed activity against *S.typh*i strains and *S.typhimurium* tested at 1000mg/ml. Eleven plant extracts were found to inhibit the growth of bacteria while 25 plant extracts were not active (Table 2). The antimicrobial potential of active extracts was evaluated according to their zone of inhibition against *Salmonella* strains, and the results (zone of inhibition) were compared with the activity of the standard, namely, Chloramphenicol and ciprofloxacin (1.0 mg/disc) (Table 3). Ethyl acetate extract of flower of *T. diversifolia* showed significant results inhibiting *S.typhi* ATCC 43579 and *S.typhi* ATCC 13347 with 18.5 ±0 mm and 18±2 mm zone of inhibition respectively when compared to the hexane extract of leaf of *T.diversifolia* activity against *S.typhi* ATCC 13347 and *S.typhi* ATCC 43579 with the zone of inhibition of 17.67±2 mm and 17±0 mm respectively. Ethyl acetate and hexane extract of *W.ugandensis* showed zone of inhibition in range of 6-9 and 6-15 mm respectively for all the *Salmonella* strains tested. Methanol extracts of *T. diversifolia* and *C. megalocapus* showed moderate and mild activity against all the tested *Salmonella* strains with zone of inhibition in range of 8-12mm and 6-8 mm respectively (Table 3). Minimum Inhibitory Concentration values of the 9 plant extracts selected based on the zone of inhibition were in the range of <0.031-15.63 mg/ml. The MIC of hexane extract of *W. ugandensis* root, *W.ugandensis* bark, *T. diversifolia* leaf and *T. diversifolia* flower fall within the following ranges: <0.031-3.91, <0.031-0.488, 0.24-1.95 and 0.12-3.91 respectively. The MIC for ethyl acetate extracts were within <0.031-0.24, <0.031-0.061, <0.031-0.98 and 0.12-15.63 respectively while MIC of methanol extract of *T. diversifolia* leaf was in range of <0.031-0.98 mg/ml (Table 4). The MIC of ciprofloxacin was between 15-25 µg/ml while chloramphenicol was within 22-30µg/ml. Preliminary phytochemical analysis revealed that the active plant extracts possessed the phytoconstitutes alkoloids, saponin, tannins, flavanoids, steroids, terpenoids and glycosides (Table 5).

## 4.0 DISCUSION

The results from the present study have shown that out of 36 extracts investigated; only eleven extracts from *W. ugendensis, T. diversifolia* and *C.megalocarpus* showed activity against 6

S*almonella* strains at 1000mg/ml while 25 plant extracts were not active. The antimicrobial potential of active extracts were evaluated according to their zone of inhibition against *Salmonella* strains, and the results (zone of inhibition) were compared with the activity of the standard, namely, chloramphenicol and ciprofloxacin (1.0 mg/disc) whose activity was in the range of 6-25 and 19-26 mm respectively. Ethyl acetate extract of flower of *T. diversifolia* showed significant results inhibiting *S.typhi* ATCC 43579 and *S.typhi* ATCC 13347 with 18.5 ±0 mm and 18±2 mm zone of inhibition respectively when compared to the hexane extract of leaf of *T.diversifolia* activity against *S. typhi* ATCC 13347 and *S. typhi* ATCC 43579 with the zone of inhibition of 17.67±2 mm and 17±0 mm respectively. Ethyl acetate and hexane extract of *W.ugandensis* showed zone of inhibition in range of 6-9 and 6-15mm respectively for *S.typhi* ATCC 13347, *S.typhi* ATCC 43579, *S.enterica* ATCC 2162, *S.typhi* ATCC 014112, *S.typhi* ATCC 01617 and *S.typhi*murium ATCC 1408 tested. Methanol extracts of *T. diversifolia* and *C. megalocapus* showed moderate and mild activity against all the tested *Solmonella* strains with zone of inhibition in range of 8-12 and 6-8 mm respectively. On the other hand, ethyl acetate extract of *W. ugandensis* root and *C. megalocapus* bark, methanol extract of *C. megalocapus* bark and ethyl acetate extract of *T. diversifolia* flower investigated against *S.typhimurium* ATCC 1408 exhibited comparatively similar activity to Chloramphenicol (6.67±0.58 mm**)** as revealed by the zone of inhibition assay. However, hexane extract of *W. ugandensis* root and *T. diversifolia* flower as well as methanol extract of *T. diversifolia* leaf were significantly higher (Table 3). The *in vitro* anti-salmonella results of this study are comparable with those from trials by Joy Hoskeri and Krishna (2011), who revealed that aqueous and methanol extracts of *Flaveria trinveria* had activity of 15.33±1.2 and 13.33±0.58 mm against *S.typhi* ATCC19430. They were however, less active than the commercially available antibacterial drug ciprofloxacin that had 21± 0.58mm (Joy Hoskeri and Krishna, 2011). It was noteworthy to compare the present investigation with what Chand, *et al*., (2008) reported. The Chand, *et al*., (2008) indicated that aqueous and methanol extracts of 47 medicinal plants studied for their anti-salmonella activity by agar well diffusion method, their result revealed that of the herbal extracts tested, 33 plant extracts were found to have activity against *S. typhi*, *S. paratyphi* A and *S. typhimurium* tested. The zones of inhibition ranged from 9 to 27mm.

Minimum Inhibitory Concentration (MIC) values of the 9 plant extracts selected based on the zone of inhibition were in the range of <0.031-15.63 mg/ml. The best hexane extracts against the *S.typhi* ATCC 13347, *S.typhi* ATCC 43579, *S.enterica* ATCC 2162 and *S.typhi*murium ATCC 1408 were those obtained from *W.ugandensis* bark with MIC values in range of <0.031-0.488 mg/ml, closely followed by *W.ugandensis* root with MIC values in the range of <0.031-3.91, then *T. diversifolia* leaf with MIC values in the range of 0.24-1.95 and the least was *T. diversifolia* flower with MIC values in the range of 0.12-3.93mg/ml. Ethyl acetate extracts of the two plants showed consistence in trend as compared with hexane extracts. The best extracts against the *Salmonella* strains was *W.ugandensis* bark, followed by *W.ugandensis* leaf, then *T. diversifolia* leaf and *T. diversifolia* flower. These extracts gave the MIC values in the range of <0.031-0.061, <0.031-0.24, <0.031-0.98 and 0.12-15.63mg/ml respectively. The methanol extract of *T. diversifolia* leaf had activity in range of <0.031-0.98 mg/ml. The MIC values of the present study are comparable with studies done by Sabiha, (2012), that demonstrated antimicrobial activity of *Thymus vulgaris* against *Salmonella typhi* in Rabbit. The MIC value of ethanol extract for *Thymus vulgaris* was determined as 2.5mg/ml (Sabiha, 2012). Also these values are in line with what Madani and Jain (2008), reported. They observed that the MIC

values of alcoholic and water extracts of *T.belerica* showed significant anti-salmonella activity and MIC was 12.5mg/ml against *S.typhimurium*.

The observed anti-salmonella activity in the present study could be attributed to the presence of a single active constituent in higher levels or due the combined effect of more than one phytoconstituent.

## CONCLUSION

*T. diversifolia* and *W.ugandensis* exert antibacteria activity against *Salmonella* strains associated with typhoid fever infection. This study therefore provides the scientific basis for its traditional application as ethnomedicine. On the other hand, the unknown components present have not been elucidated in terms of their activity and further investigations are currently ongoing on their identification and purification.

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