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In vitro study of anti-Salmonella activities of medicinal plants

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The aim of this study was to determine *in vitro* anti- *Salmonella* activity of extracts of five selected Kenyan medicinal plants against *Salmonella* ser. Typhi and *Salmonella* ser. Typhimurium. The extracts from *Tithonia diversifolia, Warburgia ugandesis, Croton megalocarpus*, *Carissa edulis* and *Launae cornuta* plants traditionally used in treatment of typhoid fever were screened for anti-*Salmonella* activity using disc diffusion and microdilution techniques. The results from the present study have shown that out of thirty six extracts investigated, only nine extracts from *T. diversifolia* and *W. ugendensis* showed activity against *Salmonella* ser. Typhi and *Salmonella* ser. Typhimurium at 1000 mg/ml. The inhibition zone of ethyl acetate, hexane and methanol extracts of *T. diversifolia* leaves, ethyl acetate and hexane extracts of *T. diversifolia* flowers, ethyl acetate and hexane extracts of *W. ugandensis* stem barks, ethyl acetate and hexane extract of *W. ugandensis* roots ranged from 8 to 18.5 ± 0 mm. These results were comparable with those of ciprofloxacin (19.67 to 26 mm) and chloramphenicol (6.67 to 24.33 mm). The minimum inhibitory concentration (MIC) of the active extracts were in the range of 0.031 to 15.63 mg/ml which compared very well with ciprofloxacin (0.015 to 0.02) and chloramphenicol (0.022 to 0.03 mg/ml). Extracts with anti-*Salmonella* activity can be used to source antibiotic substances useful in the treatment of typhoid fever. The study provides the scientific basis for the traditional application against typhoid fever.

Key words: Anti-*Salmonella* activity, medicinal plant extracts, minimum inhibitory concentration, disc diffusion technique, microdilution technique, *Salmonella* strains, typhoid.

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

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employed in the treatment and management of *Salmonella* infection. 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; 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. According to World Health Organization (WHO) more than 80% of world's population relies on traditional medicine for their primary healthcare, majority of who use plant active principles (Gupta et al., 2005). A wide variety of plants are used in Africa for treatment of fever, dysentry, cholera, diarrhoea and other infections typical of the tropical countries (Ayogu and Amadi, 2009; Ajayi and Akintola, 2010). For instance, traditional practitioners in Nigeria use herbal preparations to treat microbial infections such as typhoid and paratyphoid infections (Iroha et al., 2010).

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; Opiyo et al., 2011). The decoction from *Croton megalocarpus* bark is used as a remedy for worms and whooping cough. Grounded roots are used for syphilis, anthrax, and snakebites treatment (Kabir et al., 2005). Different communities in Africa use parts of *Carissa edulis* to alleviate pain, treat venereal diseases, glandular inflammation, induce abortion and restore virility (Githiori et al., 2004). In Kenya and Tanzania decoction from *Launae cornuta* roots is used as a remedy for cough, typhus fever and measles (Schippers, 2004).

This study investigated the anti-*Salmonella* activities of *T. diversifolia, W. ugandensis*, *C*. *megalocarpus*, *C. edulis* and *Launae cornuta*. Clinical isolates of *S.*ser.Typhi (ATCC 13347), *S.*ser.Typhi (ATCC 43579), *S.enterica* (ATCC 2162) and *S.* ser. Typhimurium (ATCC 1408) were used in the study.

MATERIALS AND METHODS

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.

Plant

The five plants selected for this study were collected in Nyamira County as indicated in Table 1. The plants were authenticated at Jomo Kenyatta University of Agriculture and Technology, Botany Department.

Experimental design

The nine plant parts obtained from 5 selected medicinal plants indicated in Table 1 were extracted using four solvent systems namely; hexane, ethyl acetate, methanol and water. The extracts obtained were subjected to standard phytochemical analyses as described by Jigna et al. (2006). In addition, the extracts were screened for anti-*Salmonella* activity against four clinical isolates. Samples in triplicate were subjected to disc diffusion and microdilition in duplicates to confirm anti-*Salmonella* activity.

Preparation of plant materials for extraction

The plant materials were washed under running tap water and left to drain off. The plant parts were chopped into small pieces. The dried pieces were ground into powder and their weights recorded.

Extraction techniques

The nine plant materials were extracted using selected solvents. Each plant material was extracted sequentially using hexane, ethyl acetate and methanol in the order of increasing polarity. Single extraction was carried out using water for each of the nine plant materials.

Preparation of hexane extract

Approximately 500 g of each plant powder was soaked separately in 1500 ml of hexane. The contents were kept for 3 days away from direct sunlight, undisturbed, then filtered through sterile filter paper. The filtrate was concentrated at 38.5 to 42°C.

Preparation of ethyl acetate extract

The hexane residues were re-soaked in 1500 ml of ethyl acetate. The contents were kept for 5 days away from direct sunlight, undisturbed and afterward filtered. The filtrate was concentrated at 38.5 to 42°C.

Preparation of methanol extract

The ethyl acetate residues were re-soaked in 1500 ml of methanol and kept for 36 h away from direct sunlight undisturbed. After filtration, the filtrate was concentrated at 65°C. The extracts were stored at 4°C until used.

Preparation of aqueous extract

Five hundred grams (500 g) of each of the powdered plant materials was weighed and soaked separately in 1500 ml of distilled water. The contents were warmed in a water bath for 2 h at

Table 1. Profile of the five medicinal plants.

Botanical name	Family name	Part of the plant used
Tithornia diversifolia	Solanaceae	Flowers and leaves
Warburgia ugandesis	Conellaceae	Roots and stem barks
Croton megalocarpus	Euphorbiaceae	Barks
Carissa edulis	Apocynaceae	Roots and barks
Launae cornuta	Asteraceae	Roots and leaves

60°C, then left to stand at room temperature for 10 h, undisturbed. They were subsequently sterile filtered and filtrate freeze dried to powder. The powder were weighed and stored until used.

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).

Controls

Water and dimethyl sulfoxide (DMSO) were used as negative controls. Ciprofloxacin and chloramphenicol (Transchem pharmaceutical Ltd, Kenya) were used as positive controls.

Disc diffusion assay

Circular paper discs (6mm diameter) were placed on Muller Hinton media inoculated with *Salmonella* strains. Sterile paper discs were dampened with 10 µl of plant extracts at 1000 mg/ml. The loaded disc was placed on the surface of the medium, the compound was allowed to diffuse for 5 min and plates were incubated for 24 h at 37°C. Discs containing ciprofloxacin and chloramphenicol were used as positive controls. Discs loaded with DMSO and water served as negative controls. The assays were performed in triplicate. Anti-*Salmonella* activity was evaluated by measuring diameter of the inhibition zone.

Determination of minimum inhibitory concentration (MIC) values

The MIC values were determined using microdilution assay as described by Eloff (1998). Ciprofloxacin and chloramphenicol were used as positive controls and DMSO was used as negative control. Plant extracts were tested against *Salmonella* strains with varying concentration ranging from 62.5 to 0.0305 mg/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 62.5 mg/ml and thereafter serially diluted plant extracts. 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, 50 µl of 5 mg/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 extract that completely suppress the growth of *Salmonella* strains.

Statistical analysis

Anti-*Salmonella* activity was determined from means of triplicates in zones of inhibition and 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.

RESULTS

Out of 36 plant extracts screened using disc diffusion assay, only nine extracts inhibited the growth of clinical *Salmonella* organisms at 1000 mg/ml. Extracts of hexane (flowers), ethyl acetate (leaves) and methanol (leaves) extracts from *T. diversifolia* were active against *S*.ser.Typhi ATCC 13347, *S*.ser.Typhi ATCC 43579, *S.enterica* ATCC 2162 and *S*.ser.Typhimurium ATCC 1408. Extracts of hexane (leaves) and ethyl acetate (flowers) from *T. diversifolia* inhibited growth of *S*.ser.Typhi ATCC13347. As was observed with ciprofloxacin and chloramphenicol controls, extracts of hexane and ethyl acetate (roots and stem bark) from *W. ugandensis* inhibited growth of all the tested *Salmonella* organisms. Extracts of methanol (leaves) from *T. diversifolia* were also observed to inhibit all the clinical isolates at 8 to 12 mm. The zones of inhibition for the active extracts are shown in Table 2.

The MIC values of the nine plant extracts was evaluated and shown to range from 0.031 to 15.63 mg/ml. The MICs of hexane extracts from *T. diversifolia* leaves and flowers ranged from 0.24 to 1.95 mg/ml and 0.12 to 3.91 mg/ml, respectively. The MICs of hexane extracts from *W*. *ugandensis* roots and stem bark ranged from 0.031 to 3.91 mg/ml and 0.031 to 0.488 mg/ml, respectively. Table 3 shows MICs of the nine plant extracts and controls. It is evident from these results that *W*. *ugandensis* extracts were the most active against all the *Salmonella* strains tested.

Table 4 illustrates anti-*Salmonella* activity values obtained from disc diffusion and microdilution methods. The nine plant extracts showed different value of anti-*Salmonella* activity against test strains. Ethyl acetate extract of *W*. *ugandensis* stem bark gave the lowest MIC value of 0.031 mg/ml against *S*.ser.Typhi ATCC 13347, *S*.ser.Typhi ATCC 43579, and *S*.ser.Typhimurium ATCC 1408 with zones of inhibition of 6, 7 and 7.33 mm,

Table 2. Zones of inhibition of clinical *Salmonella* strains by hexane, ethyl acetate and methanol extracts of selected medicinal plants

Plant extracts	Mean diameter of inhibition zones (mm)			
	***S*.ser.Typhi ATCC 13347**	***S*.ser.Typhi ATCC 43579**	***S.enterica* ATCC 2162**	***S.* ser. Typhimurium ATCC 1408**
TDLE	10 ± 0^{hij}	10 ± 0.58^{hij}	7.33 ± 0.58^{kl}	7.33 ± 2.31^{kl}
TDFH	15.67 ± 2.08^{g}	15.75 ± 1.15^{g}	7.33 ± 1.15^{kl}	6 ± 0^{l}
TDLM	11 ± 1^{h}	11.5±58h	$7.33\pm4.0.58^{kl}$	11.67 ± 0.58^{h}
TDLH	17.67 ± 2.08^{f}	17 ± 0^{f}	6 ± 0^{l}	6 ± 0^{l}
TDFE	18 ± 2^{f}	18.5 ± 0^{f}	6 ± 0^{l}	6.67 ± 0.58^{kl}
WURE	8.67 ± 0.58^{ijk}	6 ± 0^{l}	6.67 ± 0.58^{kl}	6.67 ± 1.15^{kl}
WURH	14 ± 1^{g}	8.33 ± 0.58^{jkl}	6.67 ± 1.15^{kl}	10.67 ± 4.62^{hi}
WUSBE	6 ± 0^{l}	7 ± 0^{kl}	6.33 ± 0.58^{kl}	6 ± 0^{l}
WUSBH	11 ± 3.21^{h}	7.33 ± 2.31^{kl}	6.33 ± 0.58^{kl}	7.33 ± 0.58^{kl}
DMSO(-)	6 ± 0^{l}	6 ± 0^{l}	6 ± 0^{l}	6 ± 0^{l}
CHLO(+)	23.33 ± 0.58^{de}	24 ± 1.73^{cde}	24.33 ± 0.58^{cde}	8.67 ± 0.58^{jk}
CIPRO(+)	26 ± 2^{abc}	23.33 ± 2.52^{a}	26 ± 0^{abc}	19.67 ± 1.53^{f}

IZ = Inhibition zone (in mm) includes the diameter of the disc, TDLE = *Tithonia diversifolia* leaf extract of ethyl acetate, TDLH = *Tithonia diversifolia* leaf extract of hexane, TDLM = *Tithonia diversifolia* leaf extract of methanol, TDFH = *Tithonia diversifolia* flower extract of hexane, TDFE = *Tithonia diversifolia* flower extract of ethyl acetate, WURE = *Warburgia ugandensis* root extract of ethyl acetate, WURH = *Warburgia ugandensis* root extract of hexane, WUSBE = *Warburgia ugandensis* stem bark extract of ethyl acetate, WUSBH = *Warburgia ugandensis* stem bark extract of hexane, DMSO(-)=Dimethyl sulphur dioxide (Negative control), CIPRO(+) = Ciprofloxacin (Positive control), CHLO(+) = Chloramphenicol (Positive control), Values are means of triplicate readings (Means ± SD). Means followed by different superscript letters in the table above are significantly different at $P < 0.0001$.

respectively (Table 4). The extract showed MIC of 0.061 mg/ml against *S.enterica* ATCC 2162 with inhibition zone of 6 mm. The *T. diversifolia* extracts had anti-*Salmonella* activity against all the tested clinical isolates. Methanol extract of the leaves showed activity with MIC values of 0.031, 0.24, 0.448 and 0.98 mg/ml against *S*.ser.Typhi ATCC 43579, *S*. ser. Typhi ATCC 13347, *S*. ser. Typhimurium ATCC 1408 and *S. enterica* ATCC 2162 with inhibition zones of 11.5, 11, 11.67 and 7.33, respectively.

The nine active plant extracts were screened further to study the presence of medicinally active phytochemicals in leaves, stem barks, roots and flowers. Phytochemical analysis revealed presence of alkaloids, saponin, tannins, flavanoids, steroids, terpenoids and glycosides (Table 5). Steroids were detected in all the extracts. Flavonoids and tannins were absent in hexane extracts of *T*. *diversifolia* flower and *W*. *ugandensis* stem bark. Terpenoids were found in extracts of hexane and ethyl acetate from *T*. *diversifolia* flower, *W*. *ugandensis* root and *W*. *ugandensis* stem bark. Extracts of hexane (*T*. *diversifolia* flower) and (*W*. *ugandensis* stem bark) lacked alkaloids. Glycosides were detected in extracts of hexane and ethyl acetate (*T*. *diversifolia* leaf) and ethyl acetate (*W*. *ugandensis* stem bark). Saponins were detected only in extracts of methanol from *T*. *diversifolia* leaf (Table 5).

DISCUSION

In the present study, the extracts from 5 medicinal plants were screened for activity against clinical *Salmonella* strains. Of the extracts tested, both ethyl acetate and hexane extracts of *T. diversifolia* and *W. ugandensis* exhibited activity against all four *Salmonella* strains tested in this study. Methanol extracts of *T. diversifolia* leaf also inhibited all the clinical isolates tested.

T. diversifolia plant extracts exhibited different zones of inhibition against the isolates. The ethyl acetate flower and hexane leaf extracts of *T*. *diversifolia* gave zones of inhibition at 18.5 ± 5 mm and 17.67 ± 2 mm, respectively (Table 2). This compared well with ciprofloxacin which gave zone of inhibition of 19.67 mm and therefore no significant difference in activity ($p < 0.0001$). Methanol extract of *T*. *diversifolia* leaf also showed anti-*Salmonella* activity against test isolates. The observed anti salmonella activity of *T. diversifolia* extracts agrees with the finding of Ogunfolakan et al. (2010), on broad spectrum antimicrobial activity. Kareru et al. (2010) has reported that soap made from leaf extract of *T. diversifolia* was effective against *E. coli*.

The phytochemicals and secondary metabolites from plants possess antimicrobial activity (Srikumar et al., 2007). Phytochemical analysis demonstrated the presence of alkaloids, tannin, flavonoids, terpenoids steroids and glycosides in the active extracts. Ethyl acetate flower extracts exhibited the highest anti-*Salmonella* activity. This is due to the difference in the type and concentrations of secondary metabolites in different plant parts (Srikumar et al., 2007) and may be contributing to the observed differences in anti-*Salmonela*

Table 3. Minimum Inhibitory Concentration (mg/ml) of Hexane, ethyl acetate and methanol extracts

Plant extracts	*Salmonella* organisms			
	***S.*ser.Typhi ATCC 13347 mg/ml**	***S.*ser.Typhi ATCC 43579 mg/ml**	***S.enterica* ATCC 2162 mg/ml**	***S.* ser. Typhimurium ATCC 1408 mg/ml**
TDLE	0.24^{f}	0.061^{h}	0.031^{j}	0.98^{d}
TDFH	0.98^{d}	0.12^{g}	3.91^{b}	3.91^{b}
TDLM	0.24^{f}	0.031^{j}	0.98^{d}	0.488^{e}
TDLH	0.24^{f}	0.24^{f}	1.95^{c}	0.488^{e}
TDFE	0.98^{d}	15.63^{a}	0.12^{g}	3.91^{b}
WURE	0.24^{f}	0.031^{j}	0.061^{h}	0.12^{g}
WURH	0.031^{j}	0.031^{j}	3.91^{b}	0.031^{j}
WUSBE	0.031^{j}	0.031^{j}	0.061^{h}	0.031^{j}
WUSBH	0.031^{j}	0.031^{j}	0.488^{e}	0.0467^{i}
DMSO(-)	ND	ND	ND	ND
CHLO(+)	0.022^{klm}	0.029^{jk}	0.024^{ijk}	0.030^{j}
CIPRO(+)	0.02^{lm}	0.015^{m}	0.018^{lm}	0.025^{ijk}

ND =Not defined, TDLE=*Tithonia diversifolia* leaf extract of ethyl acetate,TDFH= *Tithonia diversifolia* flower extract of hexane, TDLM= *Tithonia diversifolia* leaf extract of methanol, TDLH= *Tithonia diversifolia* leaf extract of hexane, TDFE= *Tithonia diversifolia* flower extract of ethyl acetate, WURE=*Warburgia ugandensis* root extract of ethyl acetate, WURH= *Warburgia ugandensis* root extract of hexane, WUSBE= *Warburgia ugandensis* stem bark extract of ethyl acetate, WUSBH= *Warburgia ugandensis* stem bark extract of hexane, DMSO(-VE)=Dimethyl sulphur dioxide (Negative control), CIPRO(+VE)=Ciprofloxacin(Positive control), CHLO(+VE)=Chloramphenicol(Positive control), Values are means of duplicate readings. Means followed by different superscript letters in the table above are significantly different at P<0.0001.

activity. The results of this study show that extract with high content of steroids contribute to significant inhibition of *Salmonella* growth. This finding agrees with Ashok and Vijayalakshmi (2013) who have demonstrated that sterols from *Vitis vinifera* seed exhibited antibacterial activity. The sterols could be interacting with the bacterial cell wall and membrane ultimately leading to pore formation and disrupting bacterial membrane integrity (Devjani and Barkha, 2011). Odeyemi et al. (2014) has also reported that *T. diversifolia* leaf, flower and roots extracts have antibacterial activity due to the presence of metabolic toxins such as flavonoids, steroids and alkaloids.

Hexane extracts of *W. ugandensis* roots and stem barks showed inhibition zones of 14 and 11 mm, respectively against *S*.ser.Typhi ATCC 13347. Hexane extract of *W. ugandensis* roots and chloramphenicol showed inhibition zones of 10.67 and 8.67 mm against *S*. ser. Typhimurium ATCC 1408, respectively (Table 2). This was significantly lower than that of ciprofloxacin (19.67 mm). 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 *S. 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.

The extracts of three plants namely *Croton megalocarpus, Croton edulis* and *Lactoria cornuta* had no anti-*Salmonella* activity for the extracts (Table 2). Their anti-*Salmonella* activity values were not significantly different with those of negative controls ($p < 0.0001$). The lack of anti-*Salmonella* activity in these plants may not necessarily imply the same *in vivo* since compounds may either act as pro-drug which must undergo metabolic changes to achieve the required activity. Besides, the presence of bioactive compounds depends on many factors such as the season, age, intra-species variation, part of the plant collected, soil and climate (Gessier et al., 1995).

The MIC values of the nine active plant extracts was determined. These extracts were selected because of their appreciable anti- *Salmonella* performance determined by disc diffusion. The active extracts against *Salmonella* strains were *W. ugandensis* bark, *W. ugandensis* root, *T. diversifolia* leaf and *T. diversifolia* flower. The ethyl acetate extract of *W. ugandensis* stem bark showed anti-*Salmonella* activity among the extracts tested. The extract had MIC value of 0.031 mgl/ml

Table 4. Mean anti-*Salmonella* activity values obtained by disc diffusion and microdilution technique for the active plant extracts against *Salmonella* strains.

Plant extracts		*Salmonella* organisms			
		***S*.ser.Typhi ATCC 13347**	***S*.ser.Typhi ATCC 43579**	***S.enterica* ATCC 2162**	***S.* ser. Typhimurium ATCC 1408**
TDLE	MIC (mg/ml)	0.24	0.061	0.031	0.98
	IZ (mm)	10±0	10±0.58	7.33±0.58	7.33±2.31
TDFH	MIC (mg/ml)	0.98	0.12	3.91	3.91
	IZ (mm)	15.67±2.08	15.75±1.15	7.33±1.15	6±0
TDLM	MIC (mg/ml)	0.24	0.031	0.98	0.488
	IZ (mm)	11±1	11.5±0.58	7.33±1.15	11.67±0.58
TDLH	MIC (mg/ml)	0.24	0.24	1.95	0.488
	IZ (mm)	17.67±2.08	17±0	6±0	6.67±0.58
TDFE	MIC (mg/ml)	0.98	15.63	0.12	3.91
	IZ (mm)	18±2	18.5±0	6±0	6.67±0.58
WURE	MIC (mg/ml)	0.24	0.031	0.061	0.12
	IZ (mm)	8.67±0.58	6±0	6.67±0.58	6.67±1.15
WURH	MIC (mg/ml)	0.031	0.031	3.91	0.031
	IZ (mm)	14±1	8.33±0.58	6.67±1.15	10.67±4.62
WUSBE	MIC (mg/ml)	0.031	0.031	0.061	0.031
	IZ (mm)	6±0	7±0	6.33±0.58	6±0
WUSBH	MIC (mg/ml)	0.031	0.031	0.488	0.0467
	IZ (mm)	11±3.2	7.33±2.31	6.33±0.58	7.33±0.58
DMSO(-)	MIC (mg/ml)	ND	ND	ND	ND
	IZ (mm)	6±0	6±0	6±0	6±0
CHLO(+)	MIC (mg/ml)	0.022	0.029	0.024	0.030
	IZ (mm)	23.33±0.58	24±1.73	24.33±0.58	8.67±1.53
CIPRO(+)	MIC (mg/ml)	0.02	0.015	0.018	0.025
	IZ (mm)	26±2	23.33±2.52	26±0	19.67±1.53

MIC= minimum inhibitory concentration (mg/ml), IZ=Inhibition zones (mm), TDLE=*Tithonia diversifolia* leaf extract of ethyl acetate, TDFH= *Tithonia diversifolia* flower extract of hexane, TDLM= *Tithonia diversifolia* leaf extract of methanol, TDLH= *Tithonia diversifolia* leaf extract of hexane, TDFE= *Tithonia diversifolia* flower extract of ethyl acetate, WURE=*Warburgia ugandensis* root extract of ethyl acetate, WURH= *Warburgia ugandensis* root extract of hexane, WUSBE= *Warburgia ugandensis* stem bark extract of ethyl acetate, WUSBH= *Warburgia ugandensis* stem bark extract of hexane, and CMBM= *Croton megalocarpu*s bark extract of methanol, DMSO (-VE)=Dimethyl sulphur dioxide (Negative control), CIPRO(+VE)=Ciprofloxacin(Positive control), CHLO(+VE)=Chloramphenicol(Positive control).

against *S*.ser.Typhi ATCC 13347, *S*.ser.Typhi ATCC 43579, and *S*.ser.Typhimurium ATCC 1408. It showed MIC value of 0.061 mg/ml against *S. enterica* ATCC 2162. Hexane extract of *W. ugandensis* root showed MIC value of 0.031 mg/ml against *Salmonella* strains tested except *S. enterica* ATCC 2162 which was inhibited at 3.91 mg/ml. The extracts of hexane (stem bark) and ethyl acetate (root) from *W. ugandensis* showed anti- Salmonella

Table 5. Phytochemical constituents of the active plant extracts.

Plant extracts	Alkaloids	Saponin	Tannins	Flavanoids	Steroids	Terpenoids	Glycosides
TDLE	+	-	+	+	+++	-	+
TDFH	-	-	-	-	+++	+	-
TDLM	+	++	++	+	+++	-	-
TDLH	+	-	++	+	+++	-	+
TDFE	+	-	+	+	+++	++	-
WURE	+	-	+	+	++	++	-
WURH	-	-	+	+	++	++	-
WUSBE	+	-	+	+	++	+	++
WUSBH	-	-	-	-	++	++	-

- = absent; + = present; ++ = Moderate concentration; +++ = High concentration. TDLE=*Tithonia diversifolia* leaf extract of ethyl acetate, TDFH= *Tithonia diversifolia* flower extract of hexane, TDLM= *Tithonia diversifolia* leaf extract of methanol, TDLH= *Tithonia diversifolia* leaf extract of hexane, TDFE= *Tithonia diversifolia* flower extract of ethyl acetate; WURE=*Warburgia ugandensis* root extract of ethyl acetate, WURH= *Warburgia ugandensis* root extract of hexane, WUSBE= *Warburgia ugandensis* stem bark extract of ethyl acetate, WUSBH= *Warburgia ugandensis* stem bark extract of hexane, and CMBM= *Croton megalocarpu*s bark extract of methanol.

salmonella activity against all strains tested (Table 3). Anti-*Salmonella* activity of *W. ugandensis* extracts compared well with ciprofloxacin and chloramphenicol. In a study carried out by Yibeltal et al. (2013), on antimicrobial activity of crude extracts of *W. ugandensis* against *E. coli* and *P. aeruginosa* demonstrated MIC values of 1.75 mg/ml. These results compared well with those of our study, which were in the range of 0.031 to 3.91 mg/ml (Table 3).

The methanol extract of *T. diversifolia* leaf had MIC values ranging from 0.031 to 0.98 mg/ml. The extract gave MIC values of 0.031, 0.24, 0.98 and 0.488 against *S*.ser.Typhi ATCC 43579, *S*.ser.Typhi ATCC 13347, *S*.ser.Typhimurium ATCC 1408 and *S. enterica* ATCC 2162, respectively (Table 3). Methanol and ethyl acetate extracts of *T. diversifolia* exhibited MIC values of 0.031 mg/ml each against *S*.ser.Typhi ATCC 43579 and *S.enterica* ATCC 2162, respectively. These values were not significantly different from ethyl acetate extracts of *W.ugandensis* ($p < 0.0001$). It was noted in our study that clinical *Salmonella* strains were sensitive to all *T. diversifolia* extracts at different MIC values (Table 3). Our present study has demonstrated lower MIC values for *T. diversifolia* extracts against *Salmonella* strains than what Ogundare (2007) reported. According to their report, MIC values of chloroform and methanol extracts of *T. diversifolia* were 6.25 and 3.125 mg/ml, respectively against *S. typhi*. The two extracts however gave MIC values of 6.25 mg/ml each against *P. aeruginosa*.

It was noted from this study that plant extracts tested by microdilution technique showed higher anti-*Salmonella* activity compared to values obtained from disc diffusion technique. *W. ugandensis* extracts showed lower MIC values when determined by microdilution method than by disc diffusion method. Olila et al. (2001) has reported that the paper disc retains the active component and does not allow it to diffuse into Muller Hinton agar. The paper disc is composed of cellulose [b-(1-4) linked glucose monomers]. The many free hydroxyls groups present on each glucose residues renders the surface of hydrophilic (Burgess et al., 1999). Thus, if natural products were cationic, they would be expected to adsorb to the surface of the disc and not diffuse into the medium. Consequently, a cationic polar compound displays a good antibacterial activity, but which is therefore not noticeably antibacterial by paper disc diffusion (Cleidson et al., 2007).

Most of the antibiotics used nowadays have lost their effectiveness due to development of resistant genes in microbes (Davis, 1994; Service, 1995). The antibiotics are sometimes associated with side effects such as hypersensitivity, immune suppression and allergic reaction (Ahmad et al., 1998). More interest is being shown in developing alternative antimicrobial drugs for the treatment of infectious diseases without side effects (Berahou et al., 2007; Salomao et al., 2008). The results of our present study demonstrates anti-*Salmonella* activity of *W. ugandensis* and *T diversifolia* that compared well with ciprofloxacin and chloramphenicol. The results obtained from the nine active plant extracts tested are encouraging. Further work is in progress to isolate and identify the bioactive compound(s) that could be used in the development of safer and cost effective alternative drugs for typhoid fever.

Conclusion

The results of our study showed anti-*Salmonella* activity in extracts from *W. ugandensis* and *T. diversifolia* plants. This activity compared well with that of ciprofloxacin and chloramphenicol. The study provides the basis for use of these plants in the development of drugs for management of typhoid fever.

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Conflict of interests

The author(s) have not declared any conflict of interests.

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