



Antimicrobial Activity of *Tetradenia riparia* (Hochst.) Lamiaceae, a Medicinal Plant from Tanzania

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Authors' contributions

This work was carried out between all authors. Author EAN designed the study, collected plant samples, executed laboratory experiments, performed statistical analysis, literature review and prepared the manuscript. Authors JMA and MCT supervised the microbiology part. Authors PN and JB read and corrected the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the antibacterial activity of *Tetradenia riparia* crude extracts against *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis*. The phytochemicals that are responsible for the bioactivity were also screened.

Study Design: *In vitro* assay of antibacterial properties.

Place and Duration of Study: Samples were collected from Njari village at Uru North in Moshi district located in north eastern Tanzania. Extraction and phytochemical analyses were conducted at the College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Canada. Antimicrobial assay was carried out at Department of Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada between March 2013 and August 2013.

Methodology: Agar well diffusion test was used to determine antimicrobial activity of the plant extracts. Ethanol, methanol, hexane and distilled water were used as extracting

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solvents. These extracting solvents were removed by vacuo evaporator. The resulting concentrated gummy-like materials were dissolved in Dimethylsulfoxide (10% DMSO). Chemical tests were used to determine the group of phytochemicals present in the sample extracts.

Results: Sensitivity testing results indicated that *S. aureus* was found to be more sensitive than *E. coli* and *E. faecalis*. *Tetradenia riparia* methanolic extracts from the root were the most active with zone of inhibition values of 29.33 ± 0.88 mm, 21.33 ± 0.33 mm and 20.0 ± 1.0 mm in diameter against *S. aureus*, *E. faecalis* and *E. coli* respectively. The relative inhibitory zone diameter (RIZD) was calculated. The highest percentage values of relative inhibition zone diameter of $84 \pm 5.06\%$ (*S. aureus*) and $76 \pm 6.86\%$ (*E. coli*) were demonstrated by *T. riparia* root methanolic extracts. However, *T. riparia* leaf and root extracts using hexane as well as leaf extracts using water did not show any antibacterial activity against *E. faecalis*. Root methanolic and ethanolic extracts demonstrated the minimum inhibitory concentrations ranging from 1.25mg/ml to 5.00mg/ml. Phytochemical screening of crude extracts from leaf and root of *T. riparia* revealed the presence of alkaloids, flavonoids, phenolics, saponins, tannins and sterols.

Conclusion: The study findings suggest likelihood of designing and developing potentially active antibacterial drug from *T. riparia*. Further studies should concentrate on the investigations of not only leaf but also the root part of the plant.

Keywords: *Tetradenia riparia*; *E. coli*; *S. aureus*; *E. faecalis*; phytochemicals; ethnopharmacology.

1. INTRODUCTION

Tetradenia riparia (Hochst.), commonly known as Ginger bush, is a plant species belonging to family Lamiaceae and/or Labiate [1,2]. The plant has been used for medicinal purpose by the Chagga, Pare, Meru and Maasai ethnic groups from North East regions of Tanzania. It is known as ("Ikiingiyi" or "Momboo"-in Chagga), (Ol-ikiingiyi-in Maasai and Ikingillii-in Meru). The ethnopharmacological information obtained from these ethnic groups through personal interview indicates that the plant has been used against bloody diarrhea, indigestion, constipation and malaria [3]. The fresh leaves are used to deter houseflies and mosquitoes. Moreover, Chagga, Meru and Maasai have used the leaves as tonic and are boiled with beef in meat camping feasts commonly known as (olupul) [3].

Bryant, [4] reported that leaf decoctions and infusions of *T. riparia* are widely taken for coughs and sore throats. Cold water infusion of ground leaves may also be taken for chronic coughs; this is followed by sufficient warm water to induce vomiting [4]. Leaf infusion is reported to be effective against malaria [5] and also leaf is chewed for dengue fever. The Tswana people in South Africa use leaf or shoot infusion for fever and also for gall sickness in cattle [6]. Various parts of *T. riparia* are used for treatment of boils and mumps in Kenya [5]. Leaves of *T. riparia* blended with banana and castor oil are useful as cattle medicine to treat insects [7]. Water macerates of leaves with those of *Vernonia amygdalina* Del. and *Markhamia utea* Schum are used for treatment of malaria in Rwanda [8]. *Tetradenia* plant is also used for treatment of stomach aches, mouth ulcers, toothaches, influenza and swollen legs. Inhaling the scent of crushed leaves apparently relieves headaches [9]. The plant is also used as hallucinogenic herb [10]. The strongly aromatic leaf has been reported to produce drowsiness [11]. In terms of toxicology, two cases of suspected human poisoning from self-administered over dosage of hot water extraction have been reported [11].

Antibiotics are undeniably one of the most important therapeutic discoveries of the 20th century. However, only one third of the infectious diseases known have been treated from these antibiotic synthetic products [12]. This is because of emergence of resistant pathogens that is beyond doubt the consequence of years of widespread indiscriminate use, incessant and misuse of antibiotics [13,14]. Antibiotic resistance has increased substantially in the recent years and is posing ever-increasing therapeutic problems. One of the methods to reduce the resistance to antibiotic is by using antibiotic resistance inhibitors from plants [15,16]. Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens [17]. It is expected that extracts showing target sites other than those used by antibiotics will be active against drug resistant pathogens [18]. Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. Thus, researchers have recently paid attention to safer phytomedicines and biologically active compounds isolated from plant species used as herbal medicines with acceptable therapeutic index for development of novel drugs [19].

In this study the antimicrobial efficacy of *T. riparia* was examined using methanol, ethanol, hexane and water as solvents and tested against three human pathogens *E. coli*, *S. aureus* and *E. faecalis* using well diffusion method and minimum inhibitory concentration. Although some studies of *T. riparia* have been done elsewhere, there is little information existing on the efficacy and phytochemical studies of the plant from Tanzania. The information obtained will therefore be useful in development of novel antimicrobial drugs that will help to solve the problems of antibiotic resistance on some species of bacteria and provide baseline information that is of great use for the development of pharmaceutical industries as a therapy against various human diseases.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Tetradenia riparia (Fig. 1) was collected from coffee plantation at Njari village, in Uru North, Moshi, Tanzania, near Rononi Healthy Centre (coordinates 3°16' 60"S and 37°22'0"E) about 17Km north of Moshi town in February 8th, 2013. The village where *T. riparia* plant parts were collected has typical volcanic soils and receives biannual rainfall with short rain in October to December and long rains in March to June. The plant is grown by Chagga people in their coffee home gardens (*Kihamba*) to mark boundaries and on contours where contour farming is practiced. It prefers high altitude of between 1450 up 1600m a.s.l. A voucher specimen is deposited at National Herbarium of Tanzania (NHT) at Tropical Pesticides Research Institute (TPRI)-Arusha (with reference no. EN 2980/ 2013).

Leaf, stem barks and root barks were collected, washed with tap water to remove soil debris followed by distilled water. They were then allowed to dry under shade at The Nelson Mandela African Institution of Science and Technology laboratories for 3 weeks. The plant materials were pounded to fine powder using motor and pestle, packed in cellophane papers and transported by DHL to University of Saskatchewan-Canada, School of Pharmacy and Nutrition laboratories for analysis. All research ethical issues were adhered to including material transfer agreement, sanitary and phytosanitary, plant export permit and biosafety regulation for plant material handling.



Fig. 1. *Tetradenia riparia* as found in the field at Njari village
Source: field photo

2.2 Procedure for Extraction

Powdered (100g) plant parts were weighed into four different conical flasks. To each of these flasks, 500ml of one of the following solvents was added (95% methanol, ethanol, hexane or distilled water) and then stirred by means of magnetic stirrer for 30 minutes. The mixtures were macerated under sonicator (Branson Ultrasonic 5510 OR-DTH, Alberta, Canada). Sonication was carried in water bath, at room temperature (25°C) for 30 minutes. These mixtures contained in the four conical flasks were thereafter transferred to a shaker. The shaker was set at 60rpm for 48 hours.

2.3 Filtration and Evaporation of the Sample

The extracts were filtered using Whatman® no.1 filter paper in Büchi funnel under vacuum pump. To evaporate, the samples were poured in a 250ml round bottomed flask (Pyrex® USA). Concentration by evaporation was achieved using vacuum evaporation (Büchi Vacuum V-850 Switzerland). The bath temperature was set at 45°C. Evaporation was run until a gummy like material was formed. The concentrate extracts were stored in a refrigerator at 4°C until used.

2.4 Phytochemical Analysis

Plant filtrates were prepared by boiling separately 25g of *T. riparia* leaves (TRL) and 25g of *T. riparia* root bark (TRRB) in 250ml distilled water. Thereafter, the solution was filtered in Whatman® no.1 filter paper in Büchi funnel using vacuum pump. The filtrates were used for phytochemical screening for secondary metabolites.

2.4.1 Alkaloid test

The presence of alkaloids in extracts was tested by using Wagner reagent prepared by dissolving 2g of iodine and 6g of potassium iodide in 100ml of water. Two milliliters of Wagner reagents was added to 2ml of extracts. The formations of reddish brown precipitate indicate the presence of alkaloids [20].

2.4.2 Steroids test

Briefly, acetic anhydride (2ml) was added to 5ml of extracts followed by 2ml dil. H₂SO₄. Colour change from violet to green show the presence of steroids [21].

2.4.3 Flavonoids test

The aqueous extracts filtrates (1mL) was taken in a test tube and added few drop of dilute ammonia solution. An intense yellow colour appeared in the test tube. It became colourless when 2-3 drops of dilute sulphuric acid was added that indicated the presence of flavonoids [21].

2.4.4 Test for saponins

Two grammes of the powdered sample was boiled in 20ml of distilled water in a water bath and filtered. To the filtered sample (10ml), distilled water (5ml) was added, shaken vigorously and observed for a stable persistent frothing as previously described [21].

2.4.5 Test for tannins

Dried powdered sample (0.5g) was boiled in water (20ml) in a test tube and then filtered. One milliliters of 0.1% ferric chloride (0.01 Mol/dm³) was added to 2ml of each extract sample. Brownish green colourations indicate the presence of tannins as previously described [21].

2.4.6 Anthocyanosides

One (1ml) of the plant filtrate was mixed with 2ml of dilute HCl; a pale pink color indicates the positive test [21].

2.4.7 Reducing sugars

One millilitre of the plant filtrate was mixed with Fehling A and Fehling B separately; a brown color with Fehling B and a green color with Fehling A indicate the presence of reducing sugars [21].

2.4.8 Anthraquinones

Approximately 1ml of the plant extract to be tested was shaken with 10ml of benzene and then filtered. Five millilitres of the 10% ammonia solution was then added to the filtrate and shaken. Appearance of a pink, red or violet color in the ammoniacal (lower) phase is an indication of presence of free anthraquinones [21,22].

2.5 Samples of Testing Microorganisms

The testing microorganisms were obtained from the Western College of Veterinary Medicine, Department of Microbiology-University of Saskatchewan, Canada. These bacterial samples were pure and imported from the American Type Culture Collection (ATCC) (Table 1).

Table 1. Microbial used for activity tests on *T. riparia*

Name of bacteria	Strain type	ATCC number
<i>Escherichia coli</i>	Gram-	ATCC 25922
<i>Enterococcus faecalis</i>	Gram+	ATCC 51299
<i>Staphylococcus aureus</i>	Gram+	ATCC 25923

Key: +=Gram positive bacteria; -=Gram negative bacteria

2.6 Media Preparation and Its Sterilization

For agar well diffusion assay, the method described by [23,24] was employed with modification. Antimicrobial susceptibility was tested on solid Mueller Hinton Agar (MHA) in Petri plates. For bacteria assay nutrient agar (NA) 40gm/L was used for developing surface colony growth. The suspension of culture for bacterial cells growth was done by preparing 2% Lauria broth (w/v). All the media prepared were then sterilized by autoclaving at 121°C for 20 minutes. The minimum inhibitory concentrations (MIC) values were determined by serial micro dilution assay.

2.7 Standard and Control Experiment

For the control purpose, Gentamicin® was used as a standard antibiotic while Dimethylsulfoxide (10% DMSO) without plant extract was used as a control.

2.8 Determination of Diameter of Zone of Inhibition (mm) Using Agar Well Diffusion Method

Agar well-diffusion method was employed to determine the antimicrobial activity. Eighteen-hour broth culture of the test microorganisms (Table 1) was suspended into sterile Mueller Hinton broth (MHA). It was standardized according to National Committee for Clinical Laboratory (NCCLs) by gradually adding 9% normal saline to compare its turbidity to McFarland standard of 0.5 which is approximately 1.0×10^8 (CFU/mL). Nutrient agar plates were swabbed (sterile cotton swabs) with the 18 hour old-broth culture of respective bacteria. Wells (6mm in diameter and about 4cm apart) were made in each of these plates using sterile borer. The extracts of the *T. riparia* plant were tested as follows; -Extraction of 10mg/ml of methanol, ethanol, hexane and water of *T. riparia* leaves and 10mg/ml of *T. riparia* root bark for the same solvents were tested in turn. Stock solution from the two plant parts was prepared. About 100 μ l of plant extracts were added into the wells using micropipette and allowed to diffuse at room temperature for 2 hours. Gentamicin® was used as a standard antibiotic drug in each plate. The plates were incubated at 37°C for 18-24 hours for bacterial pathogens. The diameter of the inhibition zone (mm) and diffusion rates were measured. Triplicates were maintained and the experiment was repeated thrice, for each replicate the readings were taken in three different fixed directions and the average values recorded.

2.9 Determination of the Minimum Inhibition Concentration

The minimum inhibition concentration (MIC) of plant extracts were determined according to the method previously described [25] with modification. Extract solution (concentration 20mg/ml) was serially diluted two-folds in Mueller-Hinton broth in seven test tubes to give decreasing concentration of 10mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml, 0.625mg/ml, 0.3125mg/ml and 0.156mg/ml. Gentamicin® stock concentration (2mg/ml) was serially diluted two-folds to give decreasing concentration of 1mg/ml, 0.5mg/ml, 0.25mg/ml, 0.125mg/ml, 0.0625mg/ml, 0.03125mg/ml and 0.0156mg/ml. An aliquot (0.1ml) of overnight broth culture of the test microorganism (concentration 1.5×10^8 CFU/ml) in sterile normal saline was introduced into each extract dilution. The mixtures in test tubes were incubated at 37°C for 24h. After incubation, visual turbidity was observed and recorded. Turbidity (signifying growth) or absence of it (signifying inhibition). Gentamicin® the standard antibiotic drug was used as positive control while sterile normal saline as negative control. The minimum inhibitory concentration was the lowest concentration of the extract solution that inhibited microbial growth. Results are presented in (Table 5).

2.10 Percentage Relative Inhibitory Zone Diameter for *E. coli* and *S. aureus*

Antibacterial activity was determined by measuring the inhibition zone diameter (mm) against each test organism. The antimicrobial activity expressed as percentage relative inhibition zone diameter (RIZD) was calculated according to [26] as follows:

$$\%RIZD = \left(\frac{IZD \text{ sample} - IZD \text{ negative control}}{IZD \text{ standard antibiotic}} \right) \times 100$$

RIZD is the percentage of relative inhibition zone diameter and IZD is the inhibition zone diameter (mm).

2.11 Statistical Analysis

The statistical analysis was performed using the one way analysis of variance (ANOVA) with the computations being performed with STATISTICA software program. The results are recorded as Means \pm standard error. The Fisher's least significance difference (L.S.D) was used to compare treatment means at $P=0.05$ level of significance [27].

3. RESULTS

3.1 Diameter of Zone of Inhibition (mm) Using Agar Well Diffusion Method

The inhibitory effects of different parts of *T. riparia* namely roots and leaves using different extracting solvents against the three pathogens *E. coli*, *S. aureus* and *E. faecalis* were determined. The results of zone of inhibition (ZOI) against tested pathogens are presented in (Table 2).

3.1.1 Inhibitory effects of different parts of *T. riparia* on *E. coli*

The plant extracts exhibited inhibitory effects against *E. coli* (Fig. 2). Methanol root barks extracts of *T. riparia* (TrRMeOH) demonstrated zone of inhibition value 20.0 ± 1.0 mm against *E. coli*. This was followed by root bark aqueous extracts (TrRH₂O) and leaves aqueous extracts (TrLH₂O) with values of 19.33 ± 0.33 mm and 16.0 ± 0.33 mm respectively. Leaves extract with ethanol (TrLEtOH) and root barks extract with ethanol had values of 15.66 ± 0.33 mm and 15.33 ± 0.33 mm respectively. However, Gentamicin® demonstrated the highest antimicrobial activity against *E. coli* with zone inhibition ranging from zero to 26.66 ± 0.33 mm. In contrast, *T. riparia* leaves extracted with hexane showed relatively lower antimicrobial activity with zone of inhibition value of 8.66 ± 0.33 mm against *E. coli*. The higher ZOI values for methanol and ethanol extracts may be explained in terms of solvent polarity. The plant may contain active ingredients that are more soluble in polar solvent and these were responsible for the activity.

3.1.2 Inhibitory effects of different parts of *T. riparia* on *S. aureus*

The results indicated further that, root bark extracted with methanol (TrRMeOH) had a value of 29.33 ± 0.88 mm against *S. aureus*. Followed by root barks ethanol extracts (TrREtOH) with a value of 25.66 ± 1.85 mm and leaves methanol extracts (TrLMeOH) with a value of 21.66 ± 0.33 mm. The ethanol leaves extracts (TrLEtOH) had a value of 13.0 ± 0.57 mm and root extracts in hexane (TrRHex) had a value of 12.66 ± 1.2 mm, these were relatively lower. The lowest value was given by extracts of leaves with hexane which was only 11.0 ± 0.57 mm. Gentamicin®, had the highest antimicrobial activity with zone of inhibition ranging from zero to 31.66 ± 0.66 mm. against *S. aureus* at ($P\leq 0.05$) level of significance.

3.1.3 Inhibitory effects of different parts of *T. riparia* on *E. faecalis*

The evaluation of *T. riparia* plant materials against *E. faecalis* showed that, methanol root bark extracts (TrRMeOH) demonstrated antibacterial activity with a value of 21.33 ± 0.33 mm against *E. faecalis*. It was followed by methanol extracts of leaves (TrLMeOH) with a value of 15.66 ± 0.33 mm and roots ethanol extracts which was 9.66 ± 0.88 mm. Leaves ethanol extracts gave a value of 6.66 ± 0.33 mm. The aqueous roots extracts had a value of 9.66 ± 0.33 mm. However, the extractions of leaves and root barks with hexane and those of leaves with

water had zero effects against *E. faecalis*. Likewise (Gentamicin®) had also zero inhibition against *E. faecalis*. It is worth noticing that Gentamicin®, the standard antibiotic used in this experiment had no inhibition activity against *E. faecalis*.



Fig. 2. Zone of inhibition for *E. coli* under different extracts of *T. riparia*

Source; photo taken during experiment; The numbers are 1-TrLEtOH; 2-TrLMeOH; 3-TrRMeOH; 4-TrREtOH; 5-TrRHex; 6-DMSO, GM-Gentamicin. TrLEtOH, *Tetradenia riparia* leaves with ethanol; TrLMeOH, *Tetradenia riparia* leaves with methanol; TrLHex; *Tetradenia riparia* leaves with hexane, TrRMeOH; *Tetradenia riparia* roots with methanol, TrREtOH; *Tetradenia riparia* roots with ethanol, TrRHex; *Tetradenia riparia* roots with hexane, Gentam®; Gentamicin a standard antibiotic

3.2 Percentage RIZD for *E. coli* and *S. aureus* for Different Extracts

The results of percentage relative inhibition zone diameter (RIZD) against the tested pathogens are presented in (Table 3). Methanol root barks extracts (TrRMeOH) gave the highest percentage relative inhibition zone diameter with a value ranging from zero to $76 \pm 6.86\%$ against *E. coli*. This was followed by aqueous root extracts with a value of $74 \pm 1.99\%$. Leaves extract using ethanol had a value of $63.0 \pm 3.14\%$. The lowest relative inhibitory zone diameter were given by extracts of leaves using methanol (TrLMeOH) and hexane (TrLHex) with values of $42 \pm 1.19\%$ and $37 \pm 2.38\%$ respectively against *E. coli*. On the other hand, the percentage relative inhibition zone diameter against *S. aureus* ranged from zero to $84 \pm 5.06\%$ shown by extracts of root barks in methanol (TrRMeOH). This was followed by extracts of root barks using ethanol (TrREtOH) with a value of $75 \pm 10.33\%$. The extracts of leaves using methanol (TrLMeOH) had a value of $66 \pm 6.27\%$ while extracts of

leaves using ethanol had a value of $47 \pm 3.35\%$. The lowest values were given by root barks and leaf extract using hexane with values of $40 \pm 6.4\%$ and $36 \pm 2.28\%$ respectively against *S. aureus*. It is worth noticing that Gentamicin®, experienced resistance against *E. faecalis* with inhibition of zero. The percentage relative inhibition zone diameter could not be calculated since the standard antibiotic value was zero. Additionally, other extractions such as the leaves with hexane (TrLHex), root bark with hexane (TrRHex) and the aqueous extractions of leaves (TrLH₂O) had also zero inhibition. This indicates that *E. faecalis* has higher resistance against the extractions and drug.

Table 2. Activity of plant extracts indicating (zone of inhibition, mm) on selected bacterial species

Zone of inhibition in (mm) for different bacteria species			
Extract/drug	<i>E. coli</i>	<i>S. aureus</i>	<i>E. faecalis</i>
TrLEtOH	15.66±0.33c	13.0±0.57d	6.66±0.33d
TrLMeOH	10.66±0.33d	21.66±0.33c	15.66±0.33b
TrLHex	8.66±0.33d	11.0±0.57d	0.0±0.0 e
TrLH ₂ O	16.0±0.0c	19.66±0.33c	0.0±0.0 e
TrREtOH	15.33±0.33c	25.66±1.85b	9.66±0.88c
TrRMeOH	20.0±1.0b	29.33±0.88a	21.33±0.33a
TrRHex	11.33±1.66d	12.66±1.20d	0.0±0.0 e
TrRH ₂ O	19.33±0.33b	20.0±0.57c	9.66±0.33c
Gentam	26.66±0.33a	31.66±0.66a	0.0±0.0e
DMSO	0.0±0.0e	0.0±0.0e	0.0±0.0e
One way ANOVA F statistics value			
Extract/drug	62.1***	67.4****	45.8**

Values presented are Means±SE; **, *** significant at $P \leq 0.01$, $P \leq 0.001$ respectively, ns= not significant; SE=standard error; means followed by dissimilar letter(s) in a column are significantly different from each other at $P=0.05$ according to fischer least significance difference (LSD); TrLEtOH, Tetradenia riparia leaves with ethanol; TrLMeOH, Tetradenia riparia Leaves with Methanol; TrLHex; Tetradenia riparia leaves with hexane, TrLH₂O; Tetradenia riparia leaves with water, TrRMeOH; Tetradenia riparia roots with methanol, TrREtOH; Tetradenia riparia roots with ethanol, TrRHex; Tetradenia riparia roots with hexane, Gentam®, Gentamicin a standard antibiotic

Table 3. Calculated percentage RIZD for *E. coli* and *S. aureus* for different extracts

Extract/ drug	<i>E. coli</i>	<i>S. aureus</i>
TrLEtOH	63±3.14b	47±3.35cd
TrLMeOH	42±1.19c	66±6.27b
TrLHex	37±2.38c	36±2.28d
TrLH ₂ O	62±1.19b	64±4.19bc
TrREtOH	58±1.19b	75±10.33ab
TrRMeOH	76±6.86a	84±5.06a
TrRHex	46±5.45bc	40±6.4d
TrRH ₂ O	74±1.99a	59±5.42bc
One way ANOVA (F statistics)		
Extract / drug	16.8***	8.2 ***

Values presented are Means±SE; *** significant at $P \leq 0.001$, ns=not significant; SE=standard error; means followed by dissimilar letter(s) in a column are significantly different from each other at $P=0.05$ according to fischer least significance difference (LSD). KEY: TrLEtOH, Tetradenia riparia Leaves with Ethanol; TrLMeOH, Tetradenia riparia leaves with methanol; TrLHex, Tetradenia riparia leaves with hexane; TrLH₂O, Tetradenia riparia leaves with Water; TrRMeOH, Tetradenia riparia roots with methanol; TrREtOH, Tetradenia riparia roots with ethanol; TrRHex, Tetradenia riparia roots with hexane; Gentam®, Gentamicin a standard antibiotic

3.3 Qualitative Phytochemicals Analysis of *T. riparia*

The phytochemical screening of *T. riparia* for secondary metabolites indicated the presence of the following major compounds namely alkaloids, saponins, steroids, tannins, phenols and flavonoids. Reducing sugars were also present. However, anthraquinones and anthocyanosides were absent (Table 4).

Table 4. Results of qualitative phytochemicals analysis of *T. riparia*

Phytochemical components	TRL	TRRB
Alkaloids	+	+
Anthraquinones	-	-
Anthocyanosides	-	-
Flavonoids	+	+
Phenolic	+	+
Steroids	+	+
Saponins	+	+
Tannins	+	+
Reducing sugar (with Fehling's A)	+	+
Reducing sugar (with Fehling's B)	+	+

Key: +=presence of compound in question; -=absence of the compound in question; TRL=Tetradenia riparia Leaf; TRRB=Tetradenia riparia root bark

3.4 Minimum Inhibitory Concentration

The Minimum Inhibitory concentrations (MIC) for different extracts of *T. riparia* are presented in (Table 5). The results showed that roots extracts generally had more promising activity when compared with the leaves extracts at a given concentration. The best extracts that demonstrated inhibitory effects at lower concentration include both methanol and ethanol root extracts with a value of 1.25mg/ml against *E. coli* and *S. aureus*. The ethanol and methanol leaves extracts followed with the MIC value of 2.50mg/ml against *E. coli* and *S. aureus*. In contrast the minimum inhibitory concentration that had effect against *E. faecalis* had a value of 5.00mg/ml for both root and leaves methanol and ethanol extracts.

4. DISCUSSION

In the present study, different extracts of *T. riparia* were evaluated for exploration of their antimicrobial activity against Gram positive and Gram negative bacteria. Our preliminary investigation showed that ethanol, methanol, hexane and aqueous extracts of *T. riparia* were active *in vitro* against the tested pathogens namely *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis*. Root extracts with methanol gave a value of 29.33±0.33mm zone of inhibition against *S. aureus*. This compares very closely to the standard antibiotic (Gentamicin®) with zone of inhibition of 31.66±0.66mm for the same bacterial strains. Statistically, there was no significance difference between the two values. Furthermore, root extracts with methanol had a value of 20.0±1.0mm zone of inhibition, which compares closely to the standard antibiotic (Gentamicin ®) with zone of inhibition of 26.0±0.33mm against *E. coli* at $P \leq 0.01$ level of significance. Aqueous extracts of roots had a value of 19.33±0.33mm which is closer to the standard antibiotic value. This is a good indication that a supplement antimicrobial may be developed from the root part of *T. riparia*. It is worth noting that methanol root extracts had been able to produce inhibition effect with a value of 21.33±0.33mm against *Enterococcus faecalis* whereas standard antibiotic (Gentamicin ®) encountered resistance.

Table 5. Minimal Inhibitory concentration of *T. riparia* extracts with antibacterial activity

Bacteria (Microorganisms)	Minimal inhibitory concentration of <i>T. riparia</i> extracts mg/ml								
	TrLEtOH	TrLMeOH	TrLHex	TrLH₂O	TrREtOH	TrRMeOH	TrRHex	TrRH₂O	Gentam
<i>E. coli</i>	2.50	2.50	5.00	2.50	1.25	1.25	5.00	2.50	0.125
<i>S. aureus</i>	2.50	2.50	5.00	2.50	1.25	1.25	5.00	2.50	0.125
<i>E. faecalis</i>	5.00	5.00	N/A	N/A	5.00	5.00	N/A	5.00	N/A

KEY: TrLEtOH, *Tetradenia riparia* leaves with ethanol; TrLMeOH, *Tetradenia riparia* leaves with methanol; TrLHex, *Tetradenia riparia* leaves with hexane; TrLH₂O, *Tetradenia riparia* leaves with water; TrRMeOH, *Tetradenia riparia* roots with methanol; TrREtOH, *Tetradenia riparia* roots with ethanol; TrRHex, *Tetradenia riparia* roots with hexane; Gentam®, Gentamicin a standard antibiotic; N/A, no activity observed

In a study conducted by Chow [28], it was indicated that gentamicin and vancomycin or combination was synergistically bactericidal against enterococcal strains that exhibit low-level resistance to gentamicin. *E. faecalis* resistance to Gentamicin® is explained in terms of aminoglycoside resistance [29]. There are three mechanisms of aminoglycoside resistance namely; reduced uptake or decreased cell permeability, alterations at the ribosomal binding sites, or production of aminoglycoside modifying enzymes [29,30]. Some strains of *Enterococcus* exhibit aminoglycoside resistance due to a transport defect or membrane impermeabilization [31]. This mechanism is likely chromosomally mediated and results in cross-reactivity to all aminoglycosides [32]. The present study reveals that methanol extract of *T. riparia* roots produce inhibition effects against *Enterococcus faecalis*. A comprehensive research on the extracts and phytochemicals arising from *T. riparia* leaves and roots may lead into a good source of a drug that may be developed into a suitable pharmaceutical. Though, the mechanism of action of these plant constituents is not yet fully understood it is clear that the effectiveness of the extracts largely depends on the type of solvent used. The organic extracts provide more powerful antimicrobial activity as compared with aqueous extract [26]. This observation is in line with the current study. The methanol and ethanol extracts had higher antimicrobial activity compared with the aqueous extracts against *E. coli*, *S. aureus* and *E. faecalis*. Cowan, [32] reported that most of the antibiotic compounds already identified in plants are reportedly aromatic or saturated organic molecules which can easily be solubilized in organic solvents.

Regarding percentage relative inhibitory zone, root bark extracts with methanol gave a value of $76\pm 6.86\%$ and $84\pm 5.06\%$ for *E. coli* and *S. aureus* respectively. While ethanolic root extract had a value of $75\pm 10.33\%$ against *S. aureus*. Higher percentage of relative inhibition zone diameter demonstrated by root extracts is a good indication that roots may contain higher percentages of phytochemicals that are responsible for controlling the activities of *E. coli*, *S. aureus* and *E. faecalis*. It is worth to mention here that although the indigenous knowledge and practices shows that the local people normally use leaf parts of *T. riparia* more frequently as compared with root parts for their medicinal use [3], but the findings from this study indicates that the extracts from root parts is far more effective compared with the leaves part (Table 2). Based on the current observation, I recommend that the root part of *T. riparia* should be thoroughly studied as may be a good source for a drug that may be developed into a novel antimicrobial drug.

Phytochemical screening of plant material is an important aspect, especially if there are some ethnopharmacological claims on the use of particular plant. In this regard, *T. riparia* has been reportedly used by traditional medicinal practitioners among Maasai, Chagga and Meru in treating bloody diarrhea, coughs, sores and wounds [3]. A need therefore arose to test for presence of certain secondary metabolites. The study has revealed that *T. riparia* is rich on alkaloids, phenols, steroids, saponins and tannins. The Phenolic compounds in *T. riparia* may be responsible for the therapeutic, antiseptic and antibacterial properties of the plant [33]. The observed inhibiting role on *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis* explains the reason behind the utilization of leaves in traditional medicine as anti-diarrhea, bloody diarrhea, anti-cough and treatment of general stomach aches. The mechanism of inhibitory action of these phytochemicals on microorganisms may be due to the impairments of variety of enzyme systems, including those involved in energy production, interference with the integrity of cell membrane and structural component synthesis [34,35]. Phenolic compounds are considered to be bacteriostatic against such bacteria like *E. coli* and *S. aureus* and fungistatic [35,36]. These compounds caused swelling of hyphal tips, plasma seeping around hyphae, leaking of plasma, cell wall distortions, abnormal branching or fusion of hyphae surface [34,35]. It has been observed that tannins

are responsible for anti-diarrheal activity [37] and saponins used as dietary supplements, expectorant and anti-inflammatory agents [38,39]. Alkaloids are known to possess a lot of pharmacological properties. They are mostly used as antidepressant (morphine), stimulants (caffeine), anaesthetic (cocaine), antitumor (vinblastine), antimalaria (quinine) and amoebicide (emetine) [32,40,41,]. The plant, *T. riparia* is rich in flavonoids. The biological function of flavonoids includes protection against allergies, inflammatory, free radical scavenging, platelets aggregation and microbes. Flavonoids serves as healthy promoting compounds as a result of anion radicals [36-38]. These observations support the indigenous knowledge of some ethnic groups in using this plant for treatment of skin fungal diseases as well as mouth sores [41,42]. The presence of saponins and flavonoids explains the reasons for *Tetradenia riparia* being used traditionally for treatment of bacterial related diseases.

Minimum inhibitory concentration (MIC) refers to the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation [43]. MICs are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents [43]. In the present study, the best extracts that demonstrated inhibitory effects at lower concentration included the methanol and ethanol root extracts with a value of 1.25mg/ml against *E. coli* and *S. aureus*. The ethanol and methanol leaf extracts followed with the MIC value of 2.50mg/ml. It required 5.00mg/ml against *E. faecalis* which is slightly higher compared with the amount needed for *E. coli* and *S. aureus*. This observation suggest that root parts of *T. riparia* may contain important active ingredients in reasonably small amounts that may be developed into a useful drug against *E. coli*, *S. aureus* and *E. faecalis*., thus supporting the indigenous knowledge on the use of this plant as medicine.

5. CONCLUSION

The present investigation indicates clearly that *T. riparia* contains potential antimicrobial bioactive compounds. Ethanol, methanol, hexane and aqueous extracts of *T. riparia* possess significant inhibitory effects against *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis*. Comprehensive research may lead into isolation and purification of the active ingredients that will be useful for the development of pharmaceutical as a therapy against diseases caused by the three investigated bacteria. The results of study support the indigenous knowledge of Chagga, Pare, Meru and Maasai of North Eastern Tanzania on the use of the plant as medicine along with the development of new antimicrobial drug from both root and leaf parts of this plant. Further studies should concentrate on investigations on the root part of the plant.

CONSENT

Not applicable.

ETHICAL APPROVAL

All research ethical issues were adhered to including material transfer agreement, sanitary and phytosanitary, plant export permit and biosafety regulation for bacterial and plant material handling.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Johnson CF, Phillipson PB. Reproductive biology of *Tetradenia* Benth. (Lamiaceae). In: Timberlake J, Kativus S, editors. African Plants: Biodiversity, taxonomy and uses. Royal Botanic Gardens.1999;97-104.
2. Beentje HJ. Kenya trees, shrubs and lianas. Nairobi: National Museums of Kenya; 1994.
3. Njau EA. An ethnobotanical study of medicinal plants used by Maasai people of Manyara-Arusha, Tanzania. In: Master's Thesis, School of Graduate Studies, Addis Ababa University; 2001.
4. Bryant AT. Zulu medicine and medicine-men. A Cape Town struck; 1996.
5. Githinji C, Kokwaro JO. Ethnomedicinal study of major species in the family Labiatae from Kenya. J Ethnopharmacol.1993;39:197-203.
6. Roberts M. Indigenous healing plants. Southern Book Publishers. Halfway House; 1990.
7. Van Puyvelde L, Lefebvre R, Mugabo P, de Kimpe N, Schamp N. Active principle of *Tetradenia riparia* ii antispasmodic activity of 8 (14), 15- sandaracopimaradiene-7a, 18-diol. Plant medicine.1987;52:156-158.
8. Hakizamungu E, van Puyvelde I, Wéry, M. Screening of Rwandense medicinal plants for anti-trichomonas activity. J Ethnopharmacol. 1992;36:143-146.
9. Chagnon M. General pharmacological inventory of medicinal plants of Rwanda. J of Ethnopharmacol. 1984;12(3):239-251.
10. Van Puyvelde L, Lefebvre R, Mugabo P, de Kimpe N, Schamp N. Screening of medicinal plants of Rwanda for acaricidal activity. J Ethnopharmacol. 1985;13(2):209-215.
11. Bodenstern JW. Toxicity of traditional herbal remedies. South Afr Med J. 1977;790-792.
12. Sharma A. Antibacterial activity of ethanolic extracts of some arid zone plants. Int J Pharm. Tech Res. 2011;3(1):283-286.
13. Enne VI, Livermore DM, Stephens P, Hal LMC. Persistence of sulphonamide resistance in *Escherichia coli* in the UK despite national prescribing restriction. The Lancet. 2001;28:1325-1328.
14. Westh H, Zinn CS, Rosdahl VT. An international multicenter study of antimicrobial consumption and resistance in *Staphylococcus aureus* isolates from 15 hospitals in 14 countries. Microb Drug Resist. 2004;10:169-176.
15. Kim H, Park SW, Park JM, Moon KH, Lee CK. Screening and isolation of antibiotic resistance inhibitors from herb material resistant inhibition of 21 Korean plants. Nat Prod Sci. 1995;1:50-54.
16. Alagesaboopathi C. Antimicrobial potential and phytochemical screening of *Andrographis affinis* Nees an endemic medicinal plant from India. Int J of Pharma and Pharmaceutical Sci. 2011;3(2):157-159.
17. Sen A, Batra A. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia azedarach* L. Int J Curr Pharm Res. 2012;4(2):67-73.
18. Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multiple drug resistant human pathogens. J Ethanopharmacol. 2001;74:113-123.

19. Pavithra PS, Janani VS, Charumathi KH, Indumathy R, Potala S, Verma RS. Antibacterial activity of the plant used in Indian herbal medicine. *Int J of Green Pharmacol.* 2010;10:22-28.
20. Mamta S, Jyoti S. Phytochemical screening of *Acorus calamus* and *Lantana camara*. *Int Res J Pharm.* 2012;3(5).
21. Doughari JH, Ndakidemi PA, Human IS, Benade S. Antioxidant, antimicrobial and antiverotoxic potentials of extracts of *Curtisia dentata*. *J Ethnopharmacol.* 2012;141:1040-1050.
22. Doherty VF, Olaniran OO, Kanife UC. Antimicrobial activity of *Aframomum melegueta* (Alligator pepper). *Int J Biol.* 2010;2(2).
23. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover FC, Tenover HR. *Manual of Clinical Microbiology*, 6th Ed. ASM Press, Washington DC; 1995;15-18.
24. Olurinola PF. *A laboratory manual of pharmaceutical microbiology*, Idu, Abuja, Nigeria. 1996;69-105.
25. Chung PY, Navaratnam, Chung LY. Synergistic antimicrobial activity between pentacyclic triterpenoids and antibiotics against *Staphylococcus aureus* strains. *Ann Clin Microbiol Antimicrob.* 2011;10(25).
26. Rojas JJ, Veronica JO, Saul AO, John FM. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. *BMC Comp Alt Med.* 2006;6(1):2.
27. Steel RGD, Torrie JH, Dickey DA. *Principles and procedures of statistics: A biometrical approach*. McGraw-Hill Inc. New York; 1980.
28. Chow JW. Aminoglycoside resistance in enterococci. *J Clin Infect Dis.* 2000;31:586-9.
29. Shaw KJ, Rather PN, Hare RS. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. *Microbiol Rev.* 1993;57:138-63.
30. Livermore DM, Winstanley TG, Shannon KP. Interpretative reading: Recognizing the unusual and inferring resistance mechanisms from resistance phenotypes. *J Antimicrob Chemotherapy.* 2001;48(Suppl S1):87-102.
31. Kucers A, Crowe S, Grayson ML, Hoy J, editors. *The use of antibiotics: A clinical review of antibacterial, antifungal, and antiviral drugs*. 5th ed. Oxford: Butterworth Heinemann; 1997.
32. Cowan, MM. Plant products as antimicrobial agents. *Clin Microbiol Rev.* 1999;12:564–582.
33. Huang JW, Chung WC. Management of vegetable crops diseases with plant extracts. *Advances in plant diseases management.* 2003;37:153-163.
34. Okwu DE, Morah FNI. Isolation and characterization of flavanone glycoside 4,5,7 trihydroxide flavanone rhamnoglucose from *Garcinia kola* seed. *J Appl Sci.* 2007;7(2):155-164.
35. Ali H, Dixit S. *In vitro* antimicrobial activity of flavonoids of *Ocimum sanctum* with synergistic effect of their combined form. *Asian Pac J Trop Dis.* 2012;396-398.
36. Madziga HA, Sanni S, Sandabe UK. Phytochemical and elemental analysis of *Acalypha wilkesiana* Leaf. *J Amer Sci.* 2010;6:510-514
37. Okwu DE, Igara EC. Isolation, characterization and antibacterial activity of alkaloid from *Datura metel* Linn leaves. *Afr J Pharm Pharmacol.* 2009;3:277-281.
38. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. *J Nut Biochem.* 2002;13(10):572-584
39. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasare KA. Screening of crude extracts of six medicinal plants used in South–West Nigerian unorthodox medicine for antimethicillin resistant *S. aureus* activity. *BMC Comp. Alt. Med.* 2005;5(6):1-7.

40. Karou D, Savadogo A, Canim A, Yameogo S, Montesano C, Simpore J, Colizzi V, Traore AS. Antibacterial activity of alkaloids from *Sida acuta*. Afri J Biotech. 2006;5:195-200.
41. Tanaka JCA, da Silva CC, de Oliveira AJB, Nakamura CV, Dias Filho BP. Antibacterial activity of indole alkaloids from *Aspidosperma ramiflorum*. Braz J Med Biol Res. 2006;39:387-391.
42. Akiyama H, Kazuyasu F, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action of several tannins against *Staphylococcus aureus*. J Antimicrob Chemother. 2001;48:487-491.
43. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemo. 2001;48(suppl1):5-16. doi:10.1093/jac/48.suppl_1.5. PMID 11420333.

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