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Full Length Research Paper

Antimicrobial activity and phytochemical fingerprints of five crude extracts obtained from indigenous medicinal plants of Uganda

Esther Katuura[1*], Godfrey Sande Bossa[2], Paul Waako[2] and Jasper Ogwal Okeng[2]

[1]Department of Plant Sciences, Microbiology and Biotechnology, College of Natural Sciences, Makerere University, P. O. Box 7062, Kampala, Uganda.
[2]Department of Pharmacology and Therapeutics, School of Health Sciences, Makerere University, P. O. Box 7062, Kampala Uganda.

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Five crude extracts from four Ugandan plants were screened *in vitro* for their antimicrobial activity and phytochemical composition. They included the chloroform extracts of *Bothliocline longipes, Maesa lanceolata, Trimeria bakeri, Rhus natalensis* and the petroleum ether extract of *T. bakeri*. The plant crude extracts were tested against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa (ATCC* 49619) and Entamoeba sp. Antimicrobial activities of the plants were determined by using the agar well diffusion and agar well dilution methods. The plant extracts showed activity against all the tested organisms with the zones of inhibition ranging from 4 to 19 mm. All the extracts inhibited the growth of *S. aureus* while the strongest activity was found for *T. bakeri* against *S. aureus* and Entamoeba sp. at 19 mm. Other plant extracts that induced strong antimicrobial activity were the chloroform extract of *R. natalensis* with an inhibition diameter of 13 mm against both *S. aureas* and *P. aeruginosa* and 9 mm diameter inhibition against *E. coli.* Only *T. bakeri* showed growth inhibition of *S. aureus* (4 mm). The minimum inhibitory concentration (MIC) was observed against *S. aureus* at 0.25 g/ml by the *T. bakeri* and *B. longipes* plant extracts. Sterol and triterpenes, fatty acids, flavanoids, coumarins and alkaloids were determined in *T. bakeri*, *B. longipes, R. natalensis* and *M. lanceolata.* The presence of these compounds indicates that the plants may contain an active compound or one that can be used as a template for the development of a new antimalarial or antibiotic medicine.

Key words: Antimicrobial, medicinal plants, fingerprinting, phytochemistry.

INTRODUCTION

There has been an observed steady increase in the incidence of serious secondary systemic microbial infections in many parts of the world that could cause long-lasting illnesses, increased risk of death and increased cost of health care (WHO, 2014; 2015). In addition, a strong antibiotic resistance has developed in

*Corresponding author. E-mail: katuurae@gmail.com.

almost all parts of the world (WHO, 2015). This is partially a result of the widespread use of broad-spectrum antibiotics, which eliminate or decrease the non-pathogenic microbial populations that normally compete with pathogenic ones and the application of antibiotics when they are not required (NPS, 2014). Multiple drug resistance is also increased due to the improvident use of commercial antimicrobial allopathic medicines that exist on the market (Gutman et al., 1988; FDA, 2016). In recent years, this problem is worsened as it is shown by the increased number of individuals with weakened immune responses due to the acquired immunodeficiency disease syndrome (AIDS), the action of immunosuppressant drugs or cancer chemotherapeutic agents and the poor nutrition particularly in the developing countries such as Uganda. Plants have the ability to resist against a variety of their own pathogens and can thus be considered as potential sources of different categories of compounds including antimicrobial substances (Grayer and Harborne, 1994; Harborne, 1984; Nascimento et al., 2000; Salau and Odeleye, 2007). Previously, examined plants appeared to have strong activity against various micro-organisms (Martinez et al., 1994, 1996; Khan et al., 2009). This could be one of the main reasons why herbal medicines have obtained a global acceptability.

In vitro antimicrobial properties of plants have been associated with the presence of compounds such as tannins, terpenoids, alkaloids and flavonoids (Cowan, 1999; Puupponen-Pimia, 2001). The use of phytochemicals as alternatives to drugs is widely increased (Sankar, 2016). Plants and their use in traditional health remedies are very popular among the population of Africa and are reported to have minimal side effects (Bibitha et al., 2002). In Uganda the use of herbs in alternative medicine reaches about the level of 90%, especially in rural areas (Tabuti, 2008). This fact has intensified the need for scientific data concerning the efficacy and the phytochemical properties of medicinal plants that are used in the traditional treatments. This study examined the antibacterial and antiprotozoal activities of the chloroform extracts of *Bothliocline longipes* (Oliv. & Hiern, N. E. Br., EKM 015, Asteraceae family), *Maesa lanceolata* Forssk (EKM 045, Myrsinaceae family), *Rhus natalensis* (Bernh. Ex. Krauss, Anarcadiaceae family) and *Trimeria bakeri* Gilg (EKM 019, Flacourtiace family). These plants are traditionally used by local people in treatment of various diseases and showed activity against the *P. falciparum* parasite (Katuura et al., 2007). The study also determined various phytochemical constituents that could be responsible for the antimicrobial activities against different pathogens.

MATERIALS AND METHODS

Plant collection and identification

The plant samples for laboratory analyses were collected in Nyakayojo, Mbarara district, western Uganda. Clean mature plant leaves were collected, identified and voucher specimens were deposited at Makerere University Herbarium (MHU). Samples were further transferred to the Natural Chemotherapeutic Research Institute for processing.

Processing and extraction of plant materials

The plant materials were placed on a rack in a well aerated room and dried to constant weight. The dried materials were then pounded to increase surface area to volume ratio in order to achieve maximum extraction of the active compounds. The pulverized materials (200 g) were extracted by cold maceration with occasional shaking to facilitate maximum extraction through sequential solvent removal, using petroleum ether (fraction 1), chloroform (fraction 2) and ethanol (fraction 3). The extracts were concentrated under reduced pressure using a rotary evaporator (BUCHI scientific equipments). The dry crude extracts were then stored in a fridge (4°C) until were used for antibacterial activity testing.

In vitro bacterial sensitivity tests

Water insoluble plant extracts of petroleum ether were dissolved in a few drops of dimethylsulphoxide (DMSO) and topped up with distilled water to give a stock solution of 100 mg/ml. Water soluble extracts were directly topped up to 100mg/ml with distilled water. The stock solution was kept at 4°C until required for the assay.

Preparation of the test organisms

Antimicrobial testing was done using agar well diffusion method and agar dilution techniques. *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 49619) and Entamoeba sp. were obtained from the department of Veterinary Microbiology, at the Makerere University. The organisms were first isolated on nutrient broth for 24 h and then diluted to 1: 1000 with a sterile nutrient dextrose broth. The bacterial stock solutions were used in the agar-well diffusion assays.

Preparation of media

Mueller Hinton agar was used for direct sensitivity testing. The media was prepared by adding 40 g of the agar powder to 1 ml of distilled water. The mixture was boiled and autoclaved at 121°C in a water bath. It was then transferred into sterile Petri dishes. It was allowed to set under sterile conditions, and then kept at 4°C ready for incubation.

Agar - well diffusion assay

Bacterial cultures were inoculated separately on solidified agar on each Petri dish by using a wire loop. Plant extracts (100 mg/ml) were disposed into the wells. Gentamycin (10 µg/ml) was used as a positive control. The plates were then incubated at 37°C for 24 h. The sensitivity of the test organisms to the plant extracts was determined by measuring the diameters of the zone of inhibition surrounding the wells. The diameter of growth inhibition was measured in mm.

Determination of minimum inhibitory concentration (MIC) by serial dilution method

The MIC of the plant extract was measured in extracts that caused

growth inhibition of some or all the organisms. It was determined by preparing ten dilutions of the stock extract solution in bacterial broth. Ten test tubes were arranged in a row and a serial dilution of the plant crude extracts was done with 100 mg/ml as the highest concentration. Gentamycin (10 µg/ml) was used as a positive control while the negative control did not contain the drug. The lowest concentration that inhibited growth of micro organisms was considered as the rough value of the MIC. This level was further tested as the highest value among four sets of values for the different extracts in order to obtain the actual MIC value of the tested plant extracts.

Phytochemical screening

The fleshly prepared extracts were subjected to standard qualitative phytochemical screening tests for various constituents based on procedures and basic principles by the protocols of Culei (1982) and Trease and Evans (2002). The extracts were screened for the presence of volatile oils, steroids,/triterpenoids, basic alkaloids, flavonoids, emodols/ anthracenoids, fatty acids, coumarins, tannins, alkaloid salts, reducing compounds, anthocyanins, steroid glycosides and anthroquinone glycosides among others.

Thin layer chromatography (TLC) finger printing of the plant crude extracts

The analysis was performed on chloroform crude extracts of *B. longipes*, *R. natalensis*, *M. lanceolata, T. bakeri* and petroleum ether extract of *T. bakeri*. The extracts were spotted on silica gel (Cat. no. TLC-400-525M) pre coated TLC plates (20x20 cm) with micro pipettes, 2 cm from the bottom of the plate). The TLC tank was pre-saturated with the running solvent system for 30 min before placing the spotted plate. The solvent system, methanol – xylene (1:1) was developed in order to produce unique finger prints for each extract. To run the TLC, the solvent front was allowed to move a distance of 10 cm from the spotting origin of the extracts at room temperature. The spots that were separated in each extract were detected by the use of (a) visible observation (b) UV light (c) spraying with concentrated sulphuric acid and heating in an oven for 10 min at 110°C. The R_f values (distance traveled by the spot/distance traveled by the solvent front) were measured to identify the different color properties of the compounds.

Thin layer chromatographic identification of chemical constituents in the plant extracts

Analyses were carried out on active extracts targeting specific secondary metabolites by using TLC methods reported by Harborne (1991) and Christie and Dobson (1999). Aluminium and glass TLC plates (Cat. no. TLC-400-525M & TLC-400-125F), measuring 20 cm x 20 cm, pre-coated with silica were used. A separation and identification technique, in which the solid phase moves by capillary action across a uniform thin layer of finely divided stationery phase bonded to a plate was employed (Christie and Dobson, 1999). The aluminium plate was cut into small pieces depending on the number of spots that would be run and allowed a distance of 7 to 8 cm for the movements of the various solvent systems. The spots after movement were visualized by the naked eye, under U.V light and sulphuric acid. The R_f values were determined. The Rf values and colors of the different spots were recorded in all the experiments. Sterols and triterpenes were separated in TLC using petroleum ether-dichloromethane-acetic acid (50:50:0.7) solvent system. The solvent front was allowed to run 5.8 cm and the different spots were detected using visible observation and chloroform, acetic anhydride and concentrated sulphuric acid in the ratios of 50:20:1 before heating at 120°C for 10 min (Liebermann- Burchard reaction). Flavanoids were separated using butanol-acetic acid-water (BAW) (4:1:5) solvent system. The solvent front was allowed to run 6 cm and the spots were detected using UV observation and exposure of the plates to ammonia vapor. Coumarins were separated using benzene-diethly ether (1:1) solvent system and the solvent front was allowed to run 5.5 cm. The different spots were detected using visible observation and UV light.

Column chromatography separation of compounds

The chloroform extract of *B. longipes* was separated to obtain at least one pure compound using column chromatography (Verpoorte, 1989). Gradient elution using benzene-petroleum ether-ethyl acetate solvent system was run as shown in Table 1 to separate the compounds in the extract. Preparative thin layer chromatography was employed to obtain pure compounds. Separation was achieved following the principle of gravity in a clean dry column parked with silica gel [60 (0.040-0.063 mm) Merck KGaA, 64271 Darmstadt]. The compounds were separated based on their polarities or size.

Determination of the solvent system in column chromatography

Analytical TLC was used to determine the solvent system to be used under the column. Test samples were dissolved in favorable solvent system of dissolution and introduced on TLC plates. A sample spot was introduced to the TLC plate by suction pressure using capillary tube about 1.5-2 cm from the bottom of the plate, with 1 cm separation between the samples. This was run into different solvent systems in a saturated chromatographic tank to obtain the most favorable system. R_f values 0.3-0.5 were considered for selection of solvent systems.

Preparative TLC plates

Clean dry glasses (20 x 20 cm) were coated with silica gel to make preparative TLC plates. Silica gel (60 $PF_{254 + 366}$ Merck KGaA, 64271, Darmstadt) was mixed with petroleum ether- ethyl acetate (2:1) in a ratio of 9:1, applied in form of a continuous band to the plates and modeled using coating machine (UNOPLAN, model BP. 945,868. DBGM 1, 889, 610) to make 2.5 mm thickness. The pre-coated plates were allowed to dry at room temperature over night and then activated in the oven at 60°C for 45 min. The sample of the crude extract was applied as a continuous band on the plate and run in the solvent system to separate the compounds. The different color spots of the chemical compounds were detected under UV light. The single spots were marked and scraped off from the plate and extracted three times using chloroform, which dried.

Parking of column

Dry column parking method was used. The bottom of the column was parked with a small cotton wool and filed with silica gel to about 80 cm. The column was continually tapped and left to stand for setting to be accomplished. The solvent system (mobile phase) was then added and allowed to run until it reached a consistent flow. A weighed sample of the extract was then dissolved in the required solvent system and introduced on top of the column using a beaker. The tap of the column was left open until adsorption of the sample to the silica gel was attained. A thin layer of silica gel was then applied on top of the sample in order to prevent disturbance while adding elution solvent.

Table 1. Solvent systems used in open column chromatography.

Solvent system (ratio)	Benzene	Petroleum ether	Ethyl acetate
1	0	1	0
2	0	9	1
3	0	8	2
4	0	6	4
5	0	20	80
6	40	80	30
7	40	70	40

Elution of the column

Different solvent systems were used until the different compounds of each sample were eluted. The solvent systems are described in Table 1.

Data analysis

Antimicrobial data were analyzed using SAS statistical package and the mean and least significant difference values were used to compare the antibacterial activity values of different extracts.

RESULTS

Antimicrobial activity of the plant extracts sensitivity of *M. lanceolata, T. bakeri, R. natalensis* and *B. longipes* against bacteria and protozoa *in vitro*

The result of plant extracts activity against *S. aureus* (ATCC 25923), *E. coli (*ATCC 25922), *P. aeruginosa* (ATCC 49619) and Entamoeba sp. as a summary of the zones of inhibition are shown in Table 2.

The zones of inhibition for the different plant extracts ranged from 4 to 19 mm, while growth inhibition of the microorganisms by Gentamycin ranged from 16 to 21 mm (Table 2). All the extracts were active against *S. aureus*, although the chloroform extract of *T. bakeri* exerted antimicrobial activity against all the tested organisms. The highest sensitivity was observed in *S. aureus* and Entamoeba sp. at an inhibition diameter of 19 mm. Other plant extracts that showed strong activity were the chloroform extract of *R. natalensis* with an inhibition diameter of 13 mm against both *S.* and *P. aeruginosa* and of 9 mm against *E. coli.* The petroleum extract of *T. bakeri* was active only against *S. aureus* (4 mm). The chloroform extract of *T. bakeri* had inhibition activity as Gentamycin against *P. aeruginosa* (16 mm) and Entamoeba sp. (19 mm).

Minimum inhibitory concentration (MIC)

The plant crude extracts which were tested for determination of the MIC were those which showed inhibition of the growth of the test organisms to some extent (Table 3).

Qualitative phytochemical analysis

A number of phytocompounds present in the petroleum ether, chloroform and ethanol plant extracts were determined. Some of these compounds have been previously reported to have antibacterial properties. A summary of the groups of compounds that were found present are presented in Table 4.

All the plants contained sterols/ triterpenes and fatty acids. All the plants except *R. natalensis* were found to contain flavonoids. *R. natalensis* and *B. longipes* had basic alkaloids and coumarins, in addition to the former compounds. All ethanol extracts of the plants, except *B. longipes* contained tannins, alkaloid salts, reducing compounds and steroid glycosides. Anthracenoids were also found in the ethanol extract of *T. bakeri*, and in the petroleum ether extract of *M. lanceolata.* Although the fatty acids were present in all the active plant extracts, strong positive reactions were observed in: the petroleum extract of *T. bakeri* and *B. longipes,* petroleum ether extract of *M. lanceolata*, *R. natalensis* and *B. longipes*; and in the chloroform extracts of *T. bakeri* and *R. natalensis*. Coumarins were found to be strongly positive in *B. longipes* alone; while flavonoids were positive in *T. bakeri, B. longipes* and *M. lanceolata*.

Thin layer chromatographic (TLC) separation of phytochemicals

Thin layer chromatographic separation also known as finger printing of the plant secondary metabolites was carried out to determine possible compounds present in the plant extracts. The compounds were seen as spots of different colours and R_f values under different visual systems are presented in Table 5. Specific groups of compounds were further separated and analysed. They included sterols and triterpenes (Table 6), coumarins (Table 7) and flavonoids (Table 8).

Some spots in the different plant extracts, with the

Table 2. Antimicrobial activity of the chloroform extracts of *T. bakeri, B. longipes, R. natalensis, M. lanceolata* and the petroleum ether extract of *T. bakeri.*

Organism	Agar- well medium	Zone of inhibition (mm) for different plant extracts*					
		1	2	3	4	5	6
S. aureus- ATCC 25923	Mueller-Hinton agar	19	14	13	4	5	20
E. coli- ATCC 25922	Mueller-Hinton agar	15	R	9	R	R	21
P. aeruginosa-ATCC 49619	Mueller-Hinton agar +5% sheep blood	16	R	13	R	R	16
Entamoeba sp.	Mueller-Hinton agar	19	R	R	R	R	19

Values are presented as means (mm) of triplicates. * The chloroform extracts include: 1, *T. bakeri* extracts; 2, *B. longipes* extract; 3, *R. natalensis*; 5, *M. lanceolata* extract; 4, is the petroleum ether extract of *T. bakeri*; 6. gentamycin; R, resistant.

Table 3. Minimum inhibitory concentration (MIC) of the petroleum ether extract of *T. bakeri* and the chloroform extract of *B. longipes* and *R. natalensis* against *S. aureus, E. coli, P. aeruginosa* and Enteroamoeba sp.

Organism	Extract*	Dilution (g/ml)				
		0.50	0.25	0.125	0.0625	0.06125
S. aureus	1	+	+	-	-	-
	2	+	+	-	-	-
E. coli	1	+	-	-	-	-
P. aeroginosa	1	+	-	-	-	-
	3	+	-	-	-	-
Enteroamoeba	1	+	-	-	-	-

1, T. *bakeri* extracts; 2, *B. longipes* extract; 3, *R. natalensis*. Minimum Inhibitory Concentration was 0.25 g/ml for *S. aureus* plant, *E. coli*, *P. aeruginosa* and Enteroamoeba sp. was at 0.50 g/ml (Table 3).

Table 4. Summary of the qualitative analysis of the active crude extracts of *T. bakeri, R. natalensis, B. longipes* and *M. lanceolata.*

Parameter tested	*T. Bakeri*			*R. Natalensis*			*B. longipes*			*M. lanceolata*		
	A	B	C	A	B	C	A	B	C	A	B	C
Volatile oils	-	-	*	-	-	*	-	-	*	-	-	*
Steroids/triterpenoids	++	++	*	++	++	*	++	-	*	*	+	*
Basic alkaloids	-	-	*	+	+	*	+	-	*	-	-	*
Flavonoids	-	-	+	-	*	-	+	+	*	+	+	+
Emodols/anthracenoids	-	-	+	-	-	-	-	-	*	++	-	-
Fatty acids	++	++	*	++	+	*	++	++	*	++	+	*
Coumarins	-	-	-	+	-	-	++	++	*	-	-	-
Tannins	*	*	++	*	*	++	*	*	*	*	*	++
Alkaloid salts	*	*	++	*	*	++	*	*	*	*	*	++
Reducing compound	*	*	++	*	*	++	*	*	*	*	*	++
Anthocyanin pigments	*	*	-	*	*	-	*	*	*	*	*	-
Steroid glycosides	*	*	++	*	*	++	*	*	*	*	*	++

A,Petroleum ether (PE) extract; B, Chloroform ($CHCl_3$) extract; C, Ethanol (EtOH) extract; ++, strongly positive; +, positive; -, negative; * not performed analysis.

Table 5. TLC colour spots of plant extracts versus R_f value ranges observed funder visible light, Ultra violet and sulphuric acid.

R_f value range	Plant extracts tested	Visible light	Ultra violet	Sulphuric acid
0.96-0.98	1	Yellow	Purple	Purple
	2	***Yellow***	***Green/Blue***	***Purple***
0.93-0.95	3	Yellow	Blue/Green	Purple
	4	Yellow	Red	Purple
	5	Yellow	Blue	Green
0.90-0.92	-	-	-	-
0.87-0.89	1	Blue/Green	Brown	Green
0.84-0.86	2	Blue/Green	Brown	Green
	4	Green	Red	Green
0.81-083	-	-	-	-
0.78-0.80	1	Green	***Red***	Purple
	3	-	Red	Brown
0.75-0.77	1	Green	Red	Purple
	5	Blue/Green	Brown	Green
0.72-0.74	4	Green	Red	Green
0.69-071	-	-	-	-
0.66-0.68	1	**Green**	Red	**Purple**
	4	Green	Blue	Purple
	5	Blue	Blue/Purple	Green
0.63-0.65	3	Black	Blue/Black	Purple
	5	Yellow/Green	Red	Purple
0.60-0.62	1	Yellow	Bright yellow	Yellow
	2	**Blue/Green**	Brown	Yellow/Green
	4	Green	Yellow/Green	Purple
0.57-0.59	1	Blue/ Green	Blue/ Black	*Blue*
	4	Purple	Purple	Blue
	5	Yellow	Red	Purple
0.54-0.56	2	Yellow	***Brown***	Green
	2	Blue/Black	Brown	Blue
	3	Yellow	***Red***	Green
	5	Blue/Black	Red	Blue/Black
0.51-0.53	3	Blue/Black	**Brown**	Blue
	4	Yellow	Brown	Yellow
0.48-0.50	4	Blue/Black	Red	Blue/Black
0.36-0.38	2	-	Brown	Black
0.21-0.23	2	-	Brown	Black

1, Chloroform extract of *B. longipes;* 2, chloroform extract of *T. bakeri;* 3, petroleum ether extract of *T. bakeri;* 4, chloroform extract of *M. lanceolata;* 5, chloroform extract of *R. natalensis*

Table 6. TLC evaluation of sterols and triterpenes as observed under visible light and sulphuric acid.

Plant extract	Number of spots	Rf value	Visible Light	Sulphuric acid
	1	0.99	Yellow	Green
	2	0.66	NS	Fluorescence
	3	0.52	NS	Purple
	4	0.40	Dark green	Brown /Green
1	5	0.29	Green	NS
	4	0.21	Green	NS
	6	0.19	NS	Green
	7	0.10	Yellow	NS
	8	0.03	Blue Green	Green
	1	0.40	Dark Green	NS
	2	0.29	Green	NS
2	3	0.26	Green	NS
	4	0.10	Yellow	NS
	1	0.99	Yellow	Purple
	2	0.93	Brown	Grey/Brown
	3	0.79	Yellow	Grey/Brown
	4	0.69	NS	Purple
	5	0.60	Orange	Fluorescence
	6	0.45	NS	Brown
3	7	0.40	Dark Green	Brown/Green
	8	0.34	Green	NS
	9	0.29	NS	Green
	10	0.26	Green	Green
	11	0.19	Green	Brown
	12	0.12	Yellow	Blue Black
	13	0.07	Blue Green	Green
	1	0.69	NS	Purple
	2	0.41	Dark Green	NS
	3	0.40	Dark Green	Blue Green
	4	0.34	Green	NS
4	5	0.29	NS	Green
	6	0.26	Green	Green
	7	0.19	NS	Black
	8	0.10	Yellow	Green
	9	0.05	Blue Green	Purple
	1	0.95	Yellow	Purple
	2	0.66	NS	Fluorescence
	3	0.59	Orange	NS
	4	0.48	NS	Brown
	5	0.40	Dark green	Brown Green
5	6	0.34	Green	Green
	7	0.29	NS	Green
	8	0.26	Green	Yellow
	9	0.12	NS	Blue Black
	10	0.10	Yellow	Grey

*****1, Chloroform extract of *B. longipes;* 2, chloroform extract of *T. bakeri;* 3, petroleum ether extract of *T. bakeri;* 4, chloroform extract of *M. lanceolata;* 5, chloroform extract of *R. natalensis;* T, tailing; NS, not observed.

Table 7. TLC identifications of coumarins in *B. longipes* chloroform extract.

R_f value	Colour of spots	Visible	UV
0.91	Blue	+	_
0.91	Red	_	+
0.76	Green	+	_
0.76	Fluorescent green	_	+
0.55	Brown	+	_
0.55	Violet purple	_	+
0.47	Violet purple	_	+
0.27	Blue	+	_
0.27	Fluorescent green	_	+

+, Observed colour; -, not seen or observed; UV, ultra violet.

Table 8. Detection of flavonoids in the chloroform extracts of *B. longipes* and *M. lanceolata* under UV light and Ammonia /25.

R_f Value	Colour of spots	UV		Ammonia vapour	
		B. longipes	***M. lanceolata***	***B. longipes***	***M. lanceolata***
0.83	Red	+	-	-	-
0.83	Green	-	-	+	-
0.80	Red	-	+	-	-
0.80	Green	v	-	-	+
0.67	Green	+	-	-	-
0.67	Fluorescent green	-	-	+	-
0.47	Red	-	+	-	-
0.47	Green	-	-	-	+

+, observed colour; -, not seen or observed; UV, ultra violet.

Table 9. Compounds isolated from *B. longipes.*

Compound	Color under UV	R_f	Weight (mg)
EK1	Yellow		20
EK2	Green		30
EK3	Colorless		-
EK4	Dark blue		-

same of R_f value ranges were of similar colour under the same TLC detection method. All the extracts, with R_f 0.98 – 0.93, were yellow in colour under the visible detection method, and purple after being sprayed with concentrated sulphuric acid. It was observed that some plants showed similar colours of TLC spots, under different detection methods; not withstanding their having similar R_f value ranges. For example, the chloroform extracts of *B. longipes* and *T. bakeri* at R_f value range of 0.87 to 0.89 had similar colour patterns: blue/green, brown and green when viewed under visible light, UV and sulphuric acid TLC detection methods, respectively. However, most of the plant extracts showed different colour spots under similar R_f value ranges while in others the colours of the spots were not the same under the different detection methods. For example, R_f 0.63 to 0.65 in petroleum ether extract of *T. bakeri* and the chloroform extract of *R. natalensis* were black and yellow, respectively.

Evaluation of the different types of sterols and triterpenes in the different extracts that could be active against *Plasmodium falciparum* and other test microbes showed different colour spots on TLC after spraying with acetic anhydride, chloroform and concentrated sulphuric acid using Liebermann- Burchard reaction methods was done according to Harborne (1991).

The chloroform extract of *B. longipes* had six spots: purple, blue/green, green and black in colour; and R_f values ranging from 0.69 to 0.05. The chloroform extract of *T. bakeri* had 12 spots: purple, grey/brown, fluorescence, brown, brown/green, green and blue black in colour; and R_f value ranging from 0.99 to 0.03. The chloroform fluorescent colour was observed with Liebermann-Burchard

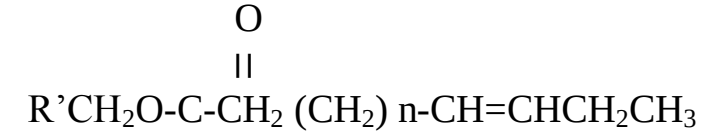

Figure 1. Compound EK2.

in some of the spots which were not observed under visible light. For the spot at R_f value 0.40, the green colour were observed: probably due to the presence of chlorophyll.

Only *B. longipes* was tested since the qualitative phytochemical test showed strong presence of coumarins in the plant extract. The extract showed four spots: blue, green and brown in colour; with R_f ranges between 0.91 and 0.27 under visible light. While the spots under UV light were five: red, fluorescent green and violet purple in colour. The spot on R_f value 0.47 was only observed under UV light as violet purple in colour.

The evaluation of flavonoids in crude extracts of *B. longipes* and *M. lanceolata*, was done using butanol acetic acid and water (BAW). The chloroform extract of *B. longipes* showed red (R_f 0.83) and green (R_f 0.67) coloured spots under UV light; whereas under ammonia vapour, the colours observed were green and fluorescent green for R_f values 0.83 to 0.67, respectively. The chroloform extract of *M. lanceolata* showed red for R_f values 0.80 and 0.47 under UV light; and green for R_f values 0.80 and 0.47 coloured spots after exposure to ammonia vapour.

The identification of the compound was performed by spectroscopy. The two most abundant extracts were characterized at pulse sequence of s2pul from spectra data and literature compound EK2 was established to be an ester characterized as shown in Figure 1.

DISCUSSION

Microbial infections remain a major concern of human health (Anneke, 2013). Many infectious microbes have shown resistance to the available medicines (WHO, 2015).In many developing countries, high child and adult mortality rates have been observed that are usually associated microbial infections (Kariuki and Dougan, 2014). Plants can offer alternative medicines with low antimicrobial resistance, wider accessibility and efficaciousness against infective microorganisms (Coelho de Souza et al., 2004). The first step towards the drug development or the herbal medicine standardization is the *in vitro* antimicrobial assays (Tona et al., 1998). Many plants have been found to have activity against more than one organism (Abedini et al., 2014). Previous researchers found proven antimicrobial activity in some of Asteraceae, Arnacardiaceae, Flarcourtiaceae and Myriscinaceae plant families (Jaime et al., 2012; Borchardt et al., 2008). This study evaluated the antimicrobial activity of selected plants that belong to these families. The chloroform extracts of *B. longipes, M. lanceolata, T. bakeri, R. natalensis* and the petroleum ether extract of *T. bakeri* inhibited the growth of the test organisms The chloroform extract of *T. bakeri* had the highest activity against the examined microbes (*S. aureus, E. coli, P. aeruginosa*, Enteroamoeba sp.). The extract showed an inhibition diameter, similar to that exerted by gentamycin, which was used as a positive control. The extract had previously been found to have antiplasmodial activity (Katuura et al., 2007). This may indicate that the extract has more than one active compound. The extract may also have synergistic or other properties such as immune boosting. Therefore a broad spectrum herbal medicine could be standardized and used in management of common microbial infections at household level especially in the rural areas of Uganda where most patients may not have access to effective allopathic antibiotics. This could also be hope for many rural patients who seek self medication without proper diagnosis. This is more so since it has been reported that many adult patients who claim to suffer from malaria, have been clinically diagnosed to have no malaria but febrile illnesses (Crump et al., 2013).

Minimum Inhibitory Concentration remains a major index of antimicrobial medicines efficacy (Gehring and Riviere, 2013). A low MIC value indicates that a lower dose of the medicine is required for inhibiting the growth of the organism; a fact that may also prevented evolution of drug-resistant microbial strains (Gullberg, 2011). In the present study, the lowest MIC was observed against *S. aureus* at 0.25 g/ml by the *T. bakeri* and *B. longipes* plant crude extracts. These plant crude extracts appeared as the most efficacious against *S. aureus*. The plant extracts can be standardized into an effective antibacterial herbal medicine or even be used as templates to develop a standard antibacterial medicine.

Many plant medicines are sometimes not efficacious due to lack of scientific knowledge to form the basis of their preparation, standardization and dosage (Kunle et al., 2012). According to previous ethnobotanical surveys (Stangeland et al., 2011; Kamatenesi-Mugisha and Oryem-Origa, 2006; Tabuti et al., 2008), the local people in Uganda mostly use water extracts in form of decoctions and for treatment of diseases. However, according to the results of this study, the chloroform ether

extract of *T. bakeri* and *R. natalensis* exhibited the highest activities. This fact partially explains why sometimes there are treatment failures amongst patients that use herbal medicines. It is necessary to improve the formulation of these medicines so that they are administered either as capsule or dry powder in order to obtain maximum absorption of the active compound by the organism *in vivo*.

Several of the molecules that possess medicinal properties have been isolated from plants (Cowan, 1999) and some are known to be effective against protozoa such as *P. falciparum,* amoeba and various bacteria (Nguedia and Shey, 2014). Phytochemical constituents such as alkaloids*,* flavonoids*,* tannins, phenols, saponins, among others serve as defense mechanism against invasion by many microorganisms, insects and other herbivores (Murugan et al., 2013). This fact may therefore explain their activity against the different microbes such as bacteria and effects on animal systems. The demonstration of antibacterial activity against gram negative, gram positive bacteria and protozoa in a plant may indicate presence of a broad spectrum of active compounds (Alam et al., 2006).

In this study, sterol and triterpenes, fatty acids, flavonoids, coumarins and alkaloids were identified in the different extracts of the *T. bakeri*, *B. longipes, R. natalensis* and *M. lanceolata* that were efficacious against various microorganisms. The presence of these compounds indicates that the examined extracts could be used as templates for the development of new anti-malarial or antibiotic medicines. The biological function of these compounds against the plasmodium parasite has been reported (Dolabela et al., 2008; Ngouamegne et al., 2008; Murata et al., 2008; Likhiwitayawuid et al., 1997; Ramanandraibe et al., 2008; Ajaiyeoba et al., 2007).

Although sterol and triterpenes, fatty acids, flavonoids, coumarins and alkaloids were qualitatively identified, their characterization and categorization in chemotaxonomic groups are necessary. Chromatographic identification and fingerprinting of the different extracts lead to the determination of the active constituents and the quality of herbal products. Although methods such as HPLC, GC, HTLC, and IR are already used, TLC is a recommended cheaper method for the development of fingerprints for specific herbal extracts or products. Authentic markers for some herbal products such as rutin, quercetin and kaempferol flavonoside, beta sistosterol sterol, beta amayrin, alpha amyrin triterpenes and nicotine, morphine and berberine alkaloids are commercially available. In some cases markers can be developed by screening the particular extracts, marking unique particular spots to the extract and measuring Rf value and color of the spot. This study lays a basis for the development of a cheap and quality control reference standard of these herbs that are used in various treatments in Uganda.

In this study, each extract was developed on TLC plates and unique color prints were exhibited. The chloroform extract of *B. longipes* had yellow or purple spot at Rf value of 0.97, yellow or bright yellow spot at Rf value of 0.61 and brown spot at Rf value of 0.59 that turned black after spraying with concentrated sulphuric acid. These spots can be used as markers for monitoring the chloroform extract of *B. longipes*. According to Harborne (1991), bright yellow spots are associated with flavonols such as kaempferol, quercetin and myricetin and pulegone, while phytosterols such as oestrone have brown color. This study lacked reference standards of the compounds. There is a need to run the TLC chromatogram of *B. longipes* chloroform extract with authentic markers. The chloroform extract of *T. bakeri* had unique grey/blue colour spot in UV, which turned purple after spraying with concentrated sulphuric acid at the Rf value of 0.95.

Different types of flavonoids separated by TLC and observed under UV light have been reported to give dull black colour with relatively low Rf values and bright yellow colour under different Rf values and solvent systems (Harborne, 1991). In his experiments, flavanols such as keaempferol, quercetin and myricetin showed bright yellow colour while gosssypetin produced dull black spots. Flavanones such as luteol, chrysoeriol and tricin also produced bright yellow colour. Isovitexin, orientin and iso-orientin glycosyl that are also flavanones produced bright yellow or yellow green and kaya flavanone biflavonyl compound produced dull black spot. Under visible light, flavonoids such as flavones were reported to be colorless and aurones yellow. Compounds such as lanciaquinones and 2,5, dihydroxy-3-(nonadec-14-enyl)-1,4-benzoquinone that are yellow brown have been reported in *M. lanceolata* extracts (Abraham et al., 2011). According to Harborne (1991), the different colour spots were developed after spraying triterpenoid with Lieberbann – Burchard reaction. Diosgenin one of the triterpenoids has been reported to undergo acid hydrolysis to dienes which normally are observed with Rf value 0.90. It is known that certain classes of chemical compounds could be used for the manufacture of specific drugs. Potential agents in plants that can be used as sources of drugs can be predicted using the principles of chemotaxonomy, such as indole alkaloids in Apocynaceae, coumarins in Rutaceae and sesuiterpene lactones in Asteraceae (Farnsworth, 1994). Plant chemical compounds with antiplasmodial activity derived from Asteraceae family include sesquieterpenes, zingiberene-3,6-β-endoperoxide and zingiberene-3,6-α-endoperoxides (Rucker et al., 1996).

Sequiterpene lactone from *Vernonia cinerea* (Chea et al., 2006), and the endoperoxide sesquiterpene lactone, artemisinin from *A. annua* (Klayman, 1985; Hien and White, 1993) are also from the same family. Artemisinin based combinations are among the best-recommended drugs for treatment of resistant Plasmodium parasites (Nosten and White, 2007). *Casearia elliptica* Willd. (L) from Flacourtiaceae family possesses antiplasmodial activity (Simonsen et al., 2001).

In this study, *B. longipes* was further analyzed for

detailed characterization of its phytochemical profile. An ester was identified among the most abundant compounds in the plant. This compound could play an important role in the formation of a new line of treatment against parasites. Probably, this genus will shed light to a class of new compounds that should be further investigated.

Conclusion

In this study, the chloroform extract of *T. bakeri* had the highest activity against the examined organisms. The extract showed a comparable activity to that of gentamycin, which was used as a positive control. The demonstration of a broad spectrum antibacterial activity of *T. bakeri* may help to discover new classes of substances that could serve as selective agents for infectious disease management. The investigation has offered the possibility of using some of these plants in drug development for microbial infections, fevers, asymptomatic malaria cases and probably for mixed infections in children. The active components of these plants however need to be characterized and further clinical trials should be carried out for scientific evidence of the above mentioned therapeutic activities in human. The differences in the antimicrobial effects of the different plant extracts could be strongly attributed to their phytochemical properties. Bioassay guided investigation should be carried out regarding the phytochemistry and pharmaceutical properties of the plants to further enrich our knowledge about their medicinal uses. The results of the present study were disseminated as a feedback to the local communities that provided the required inforation for this study. It is expected that this information will enable these communities to formulate and standardize herbal formulae that will be administered orally in form of a powder or capsule for effective management of common bacterial infections at household level.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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