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Antimicrobial Activity of *Moringa oleifera*, *Aloe vera* and *Warbugia ugandensis* on Multi-Drug Resistant *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Muhuha AW*, Kang'ethe SK and Kirira PG

Department of Medical Laboratory Sciences, Mount Kenya University, Thika, Kenya

***Corresponding author:** Muhuha AW, Department of Medical Laboratory Sciences, Mount Kenya University, Thika 342-01000, Kenya, Tel: +254723491249; E-mail: muhuhaanne@gmail.com

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Abstract

Multi-drug resistant is a global public health concern. There has been an increase in infections caused by multi-drug resistant micro-organisms in Sub Saharan Africa. This has led to extended illness, expensive health care and deaths. This experimental study was aimed to determine the anti-microbial activity of aqueous and methanol leaf extracts of *Warbugia ugandensis*, *Moringa oleifera* and *Aloe vera* on standard bacteria and multi-drug resistant clinical isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. Tetracycline drug was used as the reference drug. The bacteria were treated with extracts at different concentrations to determine the zones of inhibition through Agar Diffusion Assay, minimum inhibitory concentration and minimum bactericidal concentration assays. Raw data was analyzed using one-way and two-way analysis of variance followed by Tukey's post hoc test. Zones of inhibition ranged from 6.5 mm to 9.98 mm on the multi-drug resistant isolates, while those of the standard bacteria ranged from 6.5 mm to 12.00 mm. Methanol extracts of *W. ugandensis*, *M. oleifera* and *A. vera* at the concentration of 400 mg/ml had higher zones of inhibition against multi-drug resistant *S. aureus*, *P. aeruginosa* and *E. coli* respectively. The antimicrobial activity of the extracts indicated a concentration-dependent response. The minimum bactericidal concentration values obtained were double the minimum inhibitory concentration values. Methanol extracts recorded lower minimum inhibitory and minimum bactericidal concentrations compared to aqueous extracts. Phytochemicals which were present, included alkaloids, cardenolide glycosides, phenols, flavonoids, coumarins, tannins, saponins and anthracin glycosides. These phytochemicals are associated with antimicrobial activities. This study showed potent antimicrobial activities of methanol and aqueous extracts of *W. ugandensis*, *M. oleifera* and *A. vera* against the multi-drug resistant and standard bacteria tested. The extracts, therefore, may be used to develop alternative therapeutics in the management of multi-drug resistant *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*.

Keywords: Antimicrobial; MIC; Multi-drug resistance; MBC

Introduction

Antibiotic resistance is defined as the inability of a drug or drugs to kill a microbe that was previously used to inhibit or kill the same microbe [1]. Antimicrobial resistance is widely spread within the globe challenging the ability to manage infectious diseases that are common and thus resulting in mortality and morbidity of individuals [2]. In absence of potent antibiotics, many standard medical treatments will become ineffective against multi-drug resistant microbes [3]. This calls for the immediate global united move, otherwise, the world will head towards an era where antibiotics no longer function.

Multi-drug resistant microorganisms cause infections which are complicated and difficult to treat, examples of such microorganisms include *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. These microbes may be community or hospital acquired [4]. Multi-drug resistant *S. aureus* is accounted for causing pneumonia, intra-abdominal infection, bacteremia, osteomyelitis, food poisoning, deep tissue infection and toxic shock syndrome [5]. There are four general mechanisms that *S. aureus* use to withstand antimicrobial agents. These include drug target modification, drug uptake limitation, active efflux of the drug and inactivation of the drug [6].

Multi-drug resistant *Pseudomonas aeruginosa* causes endocarditis, pneumonia, skin and soft tissue infections, skeletal infections and bacteremia [7]. *Pseudomonas aeruginosa* is able to exhibit drug resistance through the following mechanisms; efflux pumps, mutations, multiple mutations, altered drug target and acquired resistance [8,9]. Escherichia coli commonly dominate the gut of human beings and are mostly present whenever there is fecal contamination [9]. Multi-drug resistant *E. coli* causes pneumonia, bacteremia, intra-abdominal infections, urinary tract infection (UTI), enteric infections and neonatal meningitis [9]. The resistance of E. coli is attributed to possession of phenotypes that lead to co-resistance to antibiotics from different families [10].

Plants are important natural source for products used in medicine in many years [11]. Antimicrobial activity in plant extracts is attributed to the presence of phytochemicals such as phenolics, steroids, alkaloids, saponins and terpenoids [12]. The therapeutic properties of plants have been evaluated by many studies all over the world and most of them have been revealed to possess antimicrobial activity [13-15]. Herbal preparation of *Moringa oleifera*, *Aloe vera* and *Warbugia ugandensis* are used in the management of various bacterial infections among the Kenyan communities. However, their antibacterial potential against multi-drug resistant *S. aureus*, P. aeruginosa and *E. coli* has not been scientifically documented.

Methods

Plants collection and extraction

A. vera, *M. oleifera* and *W. ugandensis* leaves were harvested from Jomo Kenyatta University of Agriculture and Technology botanical garden and identified by a taxonomist. The leaves of *W. ugandensis* and *M. oleifera* were air dried, grinded and then soaked in methanol or distilled water for one day in the ratio of 1:2 by weight volume (w/v) separately. The methanol extracts were filtered using Whatman filter paper (Whatman No. 1) and then concentrated using rotary evaporator at 64°C. The aqueous extracts were subjected to lyophilization. The fresh leaves of *A. vera* were cut into small pieces, soaked in methanol and distilled water separately for one day with occasional shaking then filtered into a clean conical flask and subjected to evaporation and lyophilization respectively. The extracts obtained were refrigerated prior to experimentation [16].

Micro-organisms

Multi-drug resistant clinical isolates (*S. aureus*, *P. aeruginosa* and *E. coli*) and standard isolates (*S. aureus* (ATCC 26923), *P. aeruginosa* (ATCC 27853) and *E. coli* (ATCC 29218)) were obtained from Microbiology National Public Health Reference Laboratory, Nairobi.

Anti-bacterial assay

The three multi-drug resistant clinical isolates (*P. aeruginosa*, *S. aureus* and *E. coli*) and the standard (control) micro-organisms (*S. aureus* (ATCC 26923), *P. aeruginosa* (ATCC 27853) and *E. coli* (ATCC 29218)) were subjected to aqueous and methanol extracts of *W. ugandensis*, *M. oleifera* and *A. vera* separately. Disc diffusion method was used to determine anti-microbial activity by measuring zones of inhibition in millimeters. A paper punch was used to prepare discs from filter papers which were kept in the hot air oven overnight at 60°C. Two-fold serial dilutions of the extracts were prepared using dimethyl sulphoxide (DMSO) for both methanol and aqueous extracts as the diluent in different test tubes. The different dilutions were used to impregnate the discs. The impregnated discs were put in the hot air oven at 50°C to dry.

The bacterial strains, both multi-drug resistant and standard (control) were suspended in physiological saline test tubes and then standardized against 0.5 McFarland to give a final density of 2.2×10^8 cfu/mL. Mueller Hinton agar was prepared and dispensed into petri dishes. Using sterile swabs, bacteria isolates were inoculated on different plates of Mueller Hinton agar and allowed to stand for thirty minutes [17]. The discs previously impregnated with extracts were placed on inoculated petri dishes using a sterile pair of forceps and incubated at 37°C overnight to determine antimicrobial activity by measuring the zones of inhibition in millimeters diameter [18].

MIC and MBC assay

To determine Minimum Inhibitory Concentration (MIC), Mueller Hinton agar was prepared and different dilutions of the extracts were dispensed into the petri dishes and were mixed thoroughly with the agar and then allowed to solidify. Different bacteria isolates were inoculated on different petri dishes with different extract dilution and incubated overnight at 37°C. The lowest concentration that inhibited the growth of bacteria was recorded as the Minimum Inhibitory Concentration (MBC) [19].

The petri dishes with no growth were swabbed using sterile swabs and inoculation done on culture plates of Mueller Hinton media, incubated overnight and observed for growth. The lowest concentration that had no observable growth was recorded as the MBC [20].

Phytochemical screening

Phytochemical screening was done according to the protocol [21,22]. The plant extracts were screened for coumarins, phenols, volatile oils, terpenoids, alkaloids, flavonoids and glycosides.

Data analysis

Raw data was entered in Microsoft Excel and later exported to Minitab version 17.0 for statistical analysis. Data was subjected to descriptive statistic and expressed as the mean ± standard error of the mean (SEM). One way ANOVA was used to determine statistical difference among different treatment groups followed by Tukey's post hoc test for pairwise comparison between different treatment groups. Two-way ANOVA was to determine the influence of different categorical independent variables on one continuous dependent variable. The p values less or equals to 0.05 were considered significant.

Results

Antibacterial activity of methanol extracts

The methanol extracts of *W. ugandensis*, *M. oliefera* and *A. vera* exhibited antibacterial activity against the standard (control) and multi-drug resistant bacteria tested (Table 1). The zones of inhibition of the three extracts at the concentration of 100, 200 and 400 mg/ml against standard bacteria were higher compared to multi-drug resistant bacteria tested (Table 1). The antibacterial activity of reference drug (tetracycline) was significantly higher compared to methanol extracts of *W. ugandensis*, *M. oleifera* and *A. vera* against the standard (control) and multi-drug resistant bacteria tested ($p<0.05$; Table 1).

The antibacterial activity of methanol extract of *M. oleifera* at the concentration of 400 mg/ml was significantly higher against standard *S. aureus* ($p<0.05$; Table 1). The methanol extract of *W. ugandensis* at the concentration of 400 mg/ml recorded the maximum zone of inhibition with a diameter of 9.89 mm against multi-drug resistant *S. aureus* (Table 1). The antimicrobial effect of methanol extracts of *W. ugandensis* at the concentrations of 200 and 400 mg/ml exhibited no significant variation against multi-drug resistant *S. aureus* and were comparable to methanol extracts of *M. oleifera* and *A. vera* at the concentration of 400 mg/ml ($p>0.05$; Table 1).

The antibacterial activities of methanol extracts of *W. ugandensis* and *M. oleifera* at the concentration of 400 mg/ml were significantly higher against standard *P. aeruginosa* ($p<0.05$; Table 1). The methanol extract of *M. oleifera* at the concentration of 400 mg/ml exhibited the highest zone of inhibition with a diameter of 8.50 mm against multi-drug resistant *P. aeruginosa*. The antibacterial effect of methanol extracts of *M. oleifera* at the concentrations of 200 and 400 mg/ml revealed no significant difference against multi-drug resistant *P. aeruginosa* and were comparable to methanol extract of *W. ugandensis* and *A. vera* at the concentration of 400 mg/ml ($p>0.05$; Table 1).

The antibacterial activity of methanol extracts of *W. ugandensis* and *A. vera* at the concentration of 400 mg/ml were significantly effective against standard *E. coli* ($p>0.05$; Table 1). The methanol extract of *A.*

vera at the concentration of 400mg/ml exhibited the highest zone of inhibition of 9.78 mm against multi-drug resistant *E. coli*. The antibacterial activity of methanol extracts at the concentrations of 200 and 400 mg/ml of *W. ugandensis* and *A. vera* were insignificant and comparable to methanol extract of *M. oleifera* at the concentration of 400 mg/ml against multi-drug resistant *E. coli* ($p>0.05$; Table 1).

Zones of inhibition (mm)						
Treatment	**Standard**	**Multidrug resistant**	**Standard**	**Multidrug resistant**	**Standard**	**Multidrug resistant**
	S. aureus	*S. aureus*	*P. aeruginosa*	*P. aeruginosa*	*E. coli*	*E. coli*
4% DMSO	0.00 ± 0.00^{g}	0.00 ± 0.00^{f}	0.00 ± 0.00^{f}	0.00 ± 0.00^{e}	0.00 ± 0.00^{f}	0.00 ± 0.00^{e}
Tetracycline	24.56 ± 0.34^{a}	19.00 ± 1.04^{a}	29.11 ± 0.39^{a}	25.33 ± 0.17^{a}	25.44 ± 0.24^{a}	21.00 ± 1.04^{a}
W. ugandansis 100 mg/ml	9.67 ± 0.33^{e}	$8.00 \pm 0.29c^{de}$	7.33 ± 0.33^{e}	6.94 ± 0.10^{d}	7.00 ± 0.00^{e}	7.44 ± 0.18^{d}
W. ugandensis 200 mg/ml	$10.00 \pm 0.00d^{e}$	$9.22 \pm 0.32b^{c}$	8.50 ± 0.29^{cd}	7.33 ± 0.17^{cd}	8.00 ± 0.00^{d}	8.44 ± 0.18^{bcd}
W. ugandensis 400 mg/ml	11.00 ± 0.00c	9.89 ± 0.11b	9.17 ± 0.17b	8.33 ± 0.17b	10.00 ± 0.00b	9.17 ± 0.12bc
M. oleifera 100 mg/ml	10.00 ± 0.00^{de}	6.94 ± 0.06^{e}	7.00 ± 0.00^{e}	6.94 ± 0.06^{d}	8.00 ± 0.00^{d}	7.56 ± 0.18^{d}
M. oleifera 200 mg/ml	11.00 ± 0.00^{c}	7.50 ± 0.17^{cde}	8.33 ± 0.17^{d}	7.89 ± 0.23^{bc}	9.00 ± 0.00^{c}	8.17 ± 0.08^{cd}
M. oleifera 400 mg/ml	12.00 ± 0.00^{b}	8.50 ± 0.24^{bcde}	9.00 ± 0.00^{bc}	8.50 ± 0.25^{b}	9.33 ± 0.33^{c}	9.00 ± 0.00^{bdc}
Aloe vera 100 mg/ml	8.67 ± 0.33^{f}	7.28 ± 0.19^{de}	7.00 ± 0.00^{e}	6.89 ± 0.07^{d}	7.33 ± 0.33^{e}	7.44 ± 0.18^{d}
Aloe vera 200 mg/ml	10.33 ± 0.33^{cde}	$7.83 \pm 0.26c^{de}$	8.17 ± 0.17^{d}	7.17 ± 0.08^{d}	8.33 ± 0.33^{d}	8.44 ± 0.18^{bcd}
Aloe vera 400 mg/ml	10.67 ± 0.33^{cd}	8.89 ± 0.26^{bcd}	9.00 ± 00^{bc}	8.06 ± 0.06^{b}	10.00 ± 0.00^{b}	9.78 ± 0.15^{b}

Values are expressed as mean ± standard error of mean (SEM) for triplicate readings. Values with the same superscript letter along the column are insignificant using one-way ANOVA followed by Tukey's post hoc test ($p>0.05$).

Table 1: Antibacterial activity of methanolic extract of *W. ugandensis*, *M. oleifera* and *A. vera* against multi drug resistant *S. aureus*, *P. aeruginosa* and *E. coli*.

Antibacterial activity of aqueous extracts

Similarly, the aqueous extract of *W. ugandensis*, *M. oleifera* and *A. vera* exhibited antibacterial activity against standard and multi-drug resistant bacteria tested (Table 2). The zones of inhibition of aqueous extract of *W. ugandensis*, *M. oleifera* and *A. vera* against standard bacteria were higher compared to multi-drug resistant bacteria at all concentrations. The antimicrobial effect of tetracycline (reference drug) was significantly higher compared to aqueous extracts of *W. ugandensis*, *M. oleifera* and *A. vera* against standard and multidrug resistant bacteria tested ($p<0.05$; Table 2).

The antibacterial effect of aqueous extracts of *W. ugandensis*, *M. oleifera* and *A. vera* at the concentrations 400 mg/ml were significantly higher against standard *S. aureus* ($p<0.05$; Table 2). The aqueous extract of *M. oleifera* at concentrations of 400 mg/ml had the maximum zone of inhibition (8.33 mm) against multi-drug resistant *S. aureus* (Table 2). The antibacterial activity of aqueous extract of *M. oleifera* at concentrations of 100, 200 and 400 mg/ml against multi-drug resistant *S. aureus* was insignificant and comparable to aqueous extract of *W. ugandensis* and *A. vera* at the concentrations of 200 and 400 mg/ml ($p>0.05$; Table 2).

The antibacterial activity of aqueous extract of *M. oleifera* at the concentration of 400 mg/ml was significantly effective against standard *P. aeruginosa* ($p<0.05$; Table 2). The aqueous extract *M. oleifera* exhibited the maximum zone of inhibition (8.5 mm) against multi-drug resistant *P. aeruginosa* at the concentration of 400 mg/ml (Table 2). The antimicrobial activity of aqueous extract of *M. oleifera* at the concentrations 400 mg/ml was significantly higher against multi-drug resistant *P. aeruginosa* ($p<0.05$, Table 2).

The antibacterial activities of aqueous extract of *M. oleifera* at the concentration of 400 mg/ml was significantly active against standard *E. coli* ($p<0.05$). The aqueous extract of *M. oleifera* at the concentration of 400mg/ml had the highest zone of inhibition of 7.89 mm against multi-drug resistant *E. coli*. The antibacterial activity of aqueous extracts of *W. ugandensis*, *M. oleifera* and *A. vera* against multi-drug resistant *E. coli* were insignificant at the concentrations of 100, 200 and 400 mg/ml respectively ($p>0.05$; Table 2). The antibacterial activity of methanol extracts of W. ugandensis, *M. oleifera* and *A. vera* against the selected multi-drug resistant bacteria strains was significantly higher compared to aqueous extracts ($p<0.05$; Table 2).

Zones of inhibition (mm)						
Treatment	**Standard**	**Multi-drug resistant**	**Standard**	**Multi-drug resistant**	**Standard**	**Multi-drug resistant**
	S. aureus	*S. aureus*	*P. aeruginosa*	*P. aeruginosa*	*E. coli*	*E. coli*
Distilled water	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{g}	0.00 ± 0.00^{h}	0.00 ± 0.00^{f}	0.00 ± 0.00^{c}
Tetracycline	24.56 ± 0.34^{a}	19.00 ± 1.04^{a}	29.11 ± 0.39^{a}	25.33 ± 0.17^{a}	25.44 ± 0.24^{a}	21.00 ± 1.04^{a}
W. ugandansis 100 mg/ml	7.00 ± 0.00^{c}	6.50 ± 0.00^{c}	6.67 ± 0.17^{f}	6.50 ± 0.00^{g}	7.00 ± 0.00^{e}	6.50 ± 0.00^{b}
W. ugandensis 200 mg/ml	7.17 ± 0.17^{c}	7.00 ± 0.00^{bc}	7.00 ± 0.00^{ef}	7.00 ± 0.00^{efg}	7.50 ± 0.00^{d}	6.94 ± 0.16^{b}
W. ugandensis 400 mg/ml	8.00 ± 0.00^{b}	7.56 ± 0.06^{bc}	7.67 ± 0.17^{cd}	7.50 ± 0.00^{cde}	8.00 ± 0.00^{c}	7.72 ± 0.19^{b}
M. oleifera 100 mg/ml	7.00 ± 0.00^{c}	7.22 ± 0.09^{bc}	7.33 ± 0.17^{cde}	7.33 ± 0.08^{cde}	7.33 ± 0.17^{de}	6.67 ± 0.08^{b}
M. oleifera 200 mg/ml	7.33 ± 0.17^{c}	7.61 ± 0.14^{bc}	7.83 ± 0.17^{c}	7.72 ± 0.12^{cd}	7.50 ± 0.00^{d}	7.56 ± 0.19^{b}
M. oleifera 400 mg/ml	8.00 ± 0.00^{b}	8.33 ± 0.14^{b}	8.50 ± 0.29^{b}	8.50 ± 0.14^{b}	8.50 ± 0.00^{b}	7.89 ± 0.22^{b}
Aloe vera 100 mg/ml	7.00 ± 0.00^{c}	6.50 ± 0.00^{c}	6.67 ± 0.17^{f}	6.78 ± 0.15^{fg}	7.00 ± 0.00^{e}	6.50 ± 0.00^{b}
Aloe vera 200 mg/ml	7.17 ± 0.17^{c}	7.00 ± 0.00^{bc}	7.17 ± 0.17^{def}	7.28 ± 0.15^{def}	7.50 ± 0.00^{d}	7.33 ± 0.17^{b}
Aloe vera 400 mg/ml	8.00 ± 0.00^{b}	7.50 ± 0.00^{bc}	7.83 ± 0.17^{c}	7.83 ± 0.19^{c}	8.00 ± 0.00^{c}	7.50 ± 0.00^{b}

Values are expressed as mean ± standard error of mean (SEM) for triplicate readings. Values with the same superscript letter in the column are insignificant using one-way ANOVA followed by Tukey's post hoc test ($p>0.05$).

Table 2: Antibacterial activity of aqueous extract of *W. ugandensis*, *M. oleifera* and *A. vera* against multi drug resistant *S. aureus*, *P. aeruginosa* and *E. coli.*

Strains	Methanol extract (mg/ml)						Aqueous extract (mg/ml)					
	W. ugandensis		*M. oleifera*		*A. vera*		*W. ugandensis*		*M. oleifera*		*A. vera*	
Bacteria strain	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Standard *S. aureus*	90	180	90	180	90	180	190	380	90	180	90	180
Multi-drug resistant *S. aureus*	90	180	90	180	90	180	160	320	90	180	90	180
Standard *P. aeruginosa*	90	180	90	180	90	180	190	380	90	180	90	180
Multi-drug resistant *P. aeruginosa*	90	180	90	180	90	180	380	≥ 400	90	180	90	180
Standard *E. coli*	90	180	90	180	90	180	190	380	90	180	90	180
Multi-drug resistant *E. coli*	160	320	160	320	160	320	380	≥ 400	160	320	260	≥400

Values expressed as mean for triplicate readings. MIC = Minimum Inhibitory Concentration; MBC= Minimum Bactericidal Concentration.

Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *W. ugandensis*, *M. oleifera* and *A. vera*.

MIC and MBC

The methanol extract of *W. ugandensis*, *M. oleifera* and *A. vera*, as well as the aqueous extracts of *M. oleifera* and *A. vera*, recorded the least MIC and MBC values of 90 and 180 mg/ml respectively against standard and multi-drug resistant S. aureus and *P. aeruginosa* (Table 3).

The methanol extract of *W. ugandensis*, *M. oleifera* and *A. vera*, as well as the aqueous extract of *M. oleifera*, had the least MIC and MBC values of 160 and 320 mg/ml respectively against multi-drug resistant *E. coli* (Table 3).

Quantitative phytochemical screening

The methanol and aqueous extracts of *Warbugia ugandensis* indicated the presence of alkaloids, cardenolide glycosides, flavonoids, coumarins, tannins, saponins and anthracin glycosides. The methanol and aqueous extracts *Moringa oleifera* revealed the presence of alkaloids, cardenolide glycosides, phenols, flavonoids, tannins, saponins. The methanol and aqueous extracts of *Aloe vera* showed the presence of alkaloids, cardenolide glycosides, coumarins, tannins (Table 4).

Phytochemical	*Warbugia ugandensis*	*Moringa oleifera*	*Aloe vera*
Alkaloids	+	+	+
Cardenolide glycosides	+	+	+
Phenols	-	+	-
Flavonoids	+	+	-
Volatile oils	-	-	-
Coumarins	+	-	+
Tannins	+	+	+
Saponins	+	+	-
Steroids	-	-	-
Anthracin glycosides	+	-	-
Presence of phytochemical is denoted by (+) sign; absence of phytochemical is denoted by (-) sign			

Table 4: Quantitative phytochemical screening.

Discussion

Resistance to antimicrobials has become a global problem [23]. There has been an estimate of 25,000 deaths in the European Union and 23,000 deaths in the United States yearly caused by antibiotic resistant bacteria [24]. The most critical group of multi-drug resistant microorganisms includes *P. aeruginosa* and *E. coli*. They are incriminated in deadly infections like pneumonia, urinary tract infections and bloodstream infections. Besides, *S. aureus* is of high priority and can cause pneumonia, urinary tract infections, toxic shock syndrome and septicemia [25]. Multi-drug resistance is a prevailing trend in the microbial world that needs to be managed either with new drugs or alternative methods of treatment [23]. Plants possess various secondary metabolites that have been discovered to have anti-microbial properties against multi-drug resistant bacteria [26-29].

The present study showed antimicrobial activity of methanol and aqueous extracts of *Warbugia ugandensis*, *Moringa oleifera* and *Aloe vera* against multi-drug resistant *S. aureus*, *P. aeruginosa* and *E. coli*. This was indicated by zones of inhibition at concentrations of 100, 200 and 400 mg/ml. Phytochemicals such as phenolics, tannins, coumarins, terpenoids, saponins and alkaloids are documented to possess antimicrobial activity [30,31]. The antimicrobial activity of *Warbugia ugandensis*, *Moringa oleifera* and *Aloe vera* may, therefore, be attributed to the presence of these bioactive compounds.

The antimicrobial properties of medicinal plants depend on specific phytochemicals, concentration, bioactive principles, antagonistic and synergistic actions [32]. Phytochemicals use different mechanisms to combat micro-organisms such as interruption of the integrity of the cell wall, damage of the cytoplasmic membrane and biofilm inhibition [33]. Phytochemicals may also act on microbiota by inhibiting the growth of microbes, interrupting some metabolic processes, interfering with signal transduction modulation, transcriptional and translational disturbances [34].

Tetracycline was used as the standard drug (positive control) against multi-drug resistant bacteria's. The antibacterial activity of tetracycline was significantly higher compared to methanol and aqueous extracts of *W. ugandensis*, *M. oleifera* and *A. vera* against all the bacterial strains tested. Tetracycline is a broad spectrum antibiotic and is able to prevent synthesis of bacterial proteins by inhibiting the association of ribosome of bacteria with amino acyl-tRNA [35].

The methanol extracts recorded higher zones of inhibitions compared to aqueous extracts. This implied that methanol was a better solvent in the extraction of bioactive with antibacterial compounds. The antibacterial activity of the extracts increased with an increase extract concentration and therefore, concentration dependent. The methanol extracts of *W. ugandensis*, *M. oleifera* and *A. vera* at the concentration of 400 mg/ml were highly effective against multi-drug resistant *S. aureus*, *P. aeruginosa* and *E. coli* respectively.

The minimum bactericidal concentration (MBC) of the extracts against multi-drug resistant *S. aureus*, *P. aeruginosa* and *E. coli* were double the minimum inhibitory concentration. This implied that the methanol and aqueous extracts of *W. ugandensis*, *M. oleifera* and *A. vera* were bacteriostatic at lower concentrations and bactericidal at higher concentrations against multi-drug resistant *S aureus*, *P. aeruginosa* and *E. coli*. However, the MBC of aqueous extract of W.

ugandensis against multi-drug resistant *P. aeruginisa* and E. coli as well as the aqueous extract of *A. vera* against multi-drug resistant *E. coli* were above the maximum concentration and, therefore, not bactericidal.

Conclusion

The methanol and aqueous extracts of *W. ugandensis*, *M. oleifera* and *A. vera* exhibited antimicrobial activity on multi-drug resistant *S. aureus*, P. aeruginosa and *E. coli* and therefore may be used as an alternative therapeutic agent in the management of clinical multi-drug resistant bacteria.

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