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Qualitative and quantitative phytochemical composition, antimicrobial activity, and brine shrimp cytotoxicity of different solvent extracts of *Acanthus polystachyus*, *Keetia gueinzii*, and *Rhynchosia elegans*

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Abstract

Background: The root, root bark, and root tubers of *Acanthus polystachyus*, *Keetia gueinzii*, and *Rhynchosia elegans* are used for managing bacterial and fungal infections among the Luo community of Kisumu East Sub County in Kenya. However, data on the efficacy of these plants against common bacterial and fungal pathogens is not available. The safety of these plants is also not known. This study aimed to investigate the phytochemical composition, antimicrobial properties, and safety of different solvent extracts of the roots, root barks, and root tubers of *Acanthus polystachyus*, *Keetia gueinzii*, and *Rhynchosia elegans*. The broth microdilution method evaluated the antimicrobial activities of the root, root bark, and root tuber extracts (water, acetone, and methanol) of *Acanthus polystachyus*, *Keetia gueinzii*, and *Rhynchosia elegans*. Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*), gram-negative (*Escherichia coli*), and fungal (*Candida albicans*) microorganisms were used in the evaluation. The safety of the extracts was evaluated in *Artemia salina*. The phytochemical composition of the extracts was determined using qualitative and quantitative assays.

Results: In general, the extracts of *Acanthus polystachyus*, *Keetia gueinzii*, and *Rhynchosia elegans* displayed poor antimicrobial properties relative to conventional antimicrobial agents including Amoxicillin, Gentamicin, and Nystatin. The aqueous extract of *Acanthus polystachyus* and the aqueous, acetone, and methanol extracts of *Keetia gueinzii* were safe in *Artemia salina* but all other extracts were cytotoxic to *Artemia salina*.

Conclusions: These findings suggest that the use of the roots, root barks, and root tubers of *Acanthus polystachyus*, *Keetia gueinzii*, and *Rhynchosia elegans* is limited by poor antimicrobial efficacy and cytotoxicity.

Keywords: Antimicrobial activity, Medicinal plants, *Acanthus polystachyus*, *Keetia gueinzii*, *Rhynchosia elegans*, *Artemia salina*

Background

Antimicrobial Resistance (AMR) is considered to be one of the major public health problems facing mankind [1]. The misuse of antimicrobial agents is a key driver of AMR [1]. Incidences of prolonged illness, the need for expensive medicines, AMR related disability and death highlight the cost of AMR to the global economy [1]. The absence of effective antimicrobial agents

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may jeopardize the success of treatment in important infections which may occur during surgery or cancer [1]. The emergence of drug resistance for clinical isolates of *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* have been reported for most of the widely prescribed antibiotics and antifungal drugs such as Amoxicillin, Gentamicin, and Nystatin [2–4]. In the last few years, there has been a global increase in the use of medicinal plants to manage both human and veterinary diseases [5]. This has prompted the World Health Organization (WHO) for the first time to add traditional medicine to the latest revision of the international statistical classification of diseases [6]. Medicinal plants may offer a range of interesting possibilities to fight drug resistance [7–9]. Their diverse pharmacological activities may be exploited for the management of various disease conditions including combating pathogens [10]. Among these plants, *Acanthus polystachyus*, *Keetia gueinzii*, and *Rhynchosia elegans* may hold some promise [11].

Acanthus polystachyus is a shrub of the Acanthaceae family, grows in the wild, and is native to several countries in East Africa including Kenya, Uganda, Tanzania, Burundi, Sudan, and Rwanda [12]. It has pink flowers and soft, hairy leaves [12]. It is traditionally indicated for malaria, scorpion sting, and as an anti-emetic [13, 14]. Studies on the wound healing activity of the leaves have also been reported [12]. *Rhynchosia elegans* is a perennial climbing herb that belongs to the Fabaceae family and is widely distributed in Eritrea, Ethiopia, Uganda, Kenya, Tanzania, the Democratic Republic of Congo, and Yemen [15]. It is traditionally indicated for malaria, common cold and fever in humans, and blackleg disease, anthrax, and amoebiasis in livestock [13, 16]. *Keetia gueinzii* is a shrub of the Rubiaceae family [17]. It has a wide geographical distribution in many African countries including Mozambique, Malawi, Zambia, Cameroon, Sudan, Ethiopia, Rwanda, Burundi, Uganda, Kenya, Tanzania, Central African Republic, and South Africa [17]. It is traditionally indicated for malaria [18]. *Acanthus polystachyus* (roots), *Keetia gueinzii* (root barks), and *Rhynchosia elegans* (root tubers) are used for managing microbial infections in Kenya [11]. There are no pharmacological reports on *Rhynchosia elegans* and *Keetia gueinzii.* Moreover, there is a paucity of information on the phytochemical composition, antimicrobial effectiveness, and safety of these plants including *Acanthus polystachyus*. The present study aimed to fill this gap by determining the phytochemical composition, antimicrobial (bacteria and fungi) activity, and brine shrimp cytotoxicity of the root, root bark, and root tuber extracts of *Acanthus polystachyus, Keetia gueinzii,* and *Rhynchosia elegans* prepared using water, acetone, and methanol.

Methods

Ethical approval

Ethical approval of the study was obtained from the Biosafety, Animal use and Ethics committee of the Faculty of Veterinary Medicine, University of Nairobi. Ref: FVM BAUEC/2019/210 and the National Commission for Science, Technology & Innovation (NACOSTI). License No: NACOSTI/P/20/3004.

Plant collection

The root bark of *Keetia gueinzii*, roots of *Acanthus polystachyus*, and root tuber of *Rhynchosia elegans* were collected in a Sub County in Kisumu with the assistance of traditional medicine practitioners in the study area. The plant samples were identified at the University Herbarium and voucher specimens deposited for future reference; JM2019/264/001 for root bark of *Keetia gueinzii*, JM2019/284/003 for roots of *Acanthus polystachyus*, and JM2019/284/002 for root tubers of *Rhynchosia elegans*.

Preparation of plant material

Plant parts were washed with running water and allowed to dry. The root bark of *Keetia gueinzii* was scraped while the whole root of *Acanthus polystachyus* and root tuber of *Rhynchosia elegans* were cut into small pieces. The parts were dried in air at room temperature for 4 weeks and ground to a fine powder weighed on an analytical balance, and stored in zip-lock plastic bags awaiting further use.

Extraction

Aqueous extract

The method of Tadesse et al. and Yong et al. were used with modifications [19, 20]. Briefly, one hundred and sixty-five grams of sample was weighed into a 2-L conical flask and 1-L of distilled water added until there was complete dissolution. The conical flask was placed in a water bath at 90^0 C and incubated for 60 min with intermittent shaking. The resulting solution was filtered, supernatant collected into 1-L flasks, and freeze-dried using a Buchi Lyovapor freeze drier operating at 0.5 mbar and − 104 °C. The recovered powder was transferred into an air-tight glass container until further use [19, 20]. The percentage yield of the extract was calculated.

Organic extracts (acetone and methanol)

The methods of Mostafa et al. and Naz et al. were used with modifications [21, 22]. Briefly, five hundred grams of the powdered plant materials were separately macerated with 100% acetone and 100% methanol for 72 h with intermittent shaking. These were first filtered through cotton wool to remove coarse residues then further filtered through a Whatman No. 1 filter paper and

centrifuged at 5000 rpm for 10 min. The resulting filtrates were concentrated on a Buchi-R Rota-vapor under reduced pressure at 50 °C. The percentage yield of the extracts was calculated.

Qualitative phytochemical screening

The methods described by Iqbal et al., Pandey and Jaradat et al. were used to evaluate the presence of alkaloids, phenols, saponins, flavonoids, glycosides, terpenoids, and tannins in the prepared extracts [23–26]. Visual color changes/precipitation was used for evaluation and qualitatively recorded as + to indicate the presence of a metabolite and − to indicate the absence of a metabolite.

Quantitative phytochemical composition

Total phenolic content determination

The phenolic content of the extracts was determined using the methods of Harnafi et al. and Singleton et al. [27, 28] with modifications as described by Okumu et al. [29]. The standard which was used to represent phenolics in the extracts was Gallic acid. The standard (Gallic acid; 10 mg) was dissolved in 100 mL of methanol. Serial dilutions ranging from 0.25 to 2.0 were pipetted from the prepared standard solution and transferred to 10 mL volumetric flasks. 2.5 mL of a 1:10 dilution of Folin reagent and 2.0 mL of Sodium carbonate (Na_2CO_3; 7.5% w/v) solution was added. Distilled water was added to the mixture until the total volume in the flask was 10 mL. Distilled water + Folin reagent + Na_2CO_3 served as the blank. All flasks were kept on a water bath at 45 °C for ¼ of an hour. Spectroscopic readings were taken at 765 nm. The readings were used to calibrate a standard curve. The extracts under investigation were weighed (10 mg) and dissolved in 10 mL of methanol. A pipette was used to transfer one mL of the extract + methanol mixture to a 10 mL volumetric flask and the colour was developed in a similar manner to the standard. Spectroscopic readings (in triplicate) of the different extracts were taken at 765 nm. The results were summarized as mean ± standard deviation (Mean ± SD) and the quantities of phenolics in the test samples were determined by extrapolation from the curve and calculated as milligrams of Gallic acid equivalents per gram of the dry plant material (mg. GAE.g-1). The formula described by Gouveia and coworkers was used [30], i.e.

$$\text{Total phenolic content in mg/g} = \text{Concentration of Gallic acid in mg/mL} \times \text{Volume of extract solution in mL} / \text{weight of the extract in grams (g)}$$

Total flavonoid content determination

The flavonoid content of the prepared extracts was determined according to the method of Atanassova et al. [31] with modifications as described by Okumu et al. [29]. The standard used to represent flavonoids in the extracts was Catechin. The standard (10 mg) was dissolved in 100 mL of methanol to make a stock solution. Aliquots of 0.1 to 1.0 mL were pipetted from the stock solution and transferred to 10 mL volumetric flasks which contained distilled water (4 mL). Sodium Nitrite (NaNO2); 0.3 mL (5% w/v) was added. 10% w/v Aluminum Chloride ($AlCl_3$); 0.3 mL was added after 5 min. 1 M Sodium hydroxide (NaOH; 2 mL was added after a further 6 min and the total volume was made up to the mark using distilled water. Spectroscopic reasings were taken at 510 nm. Distilled water was used to blank the instrument. A calibration curve was prepared from the spectroscopic readings. Extracts were weighed (10 mg) and dissolved in 10 mL methanol. An aliquot (1 mL) of each of the extracts was pipetted to 10 mL volumetric flasks and treated in the same manner as the standard. Spectroscopic readings were taken at 510 nm. Distilled water was used to blank the instrument. All tests were done in triplicate and the results averaged and expressed as mean ± standard deviation (Mean ± SD). The concentration of flavonoids in the extracts was determined by extrapolation from the curve and calculated as milligrams of Catechin equivalents per gram of the dry plant material (mg. CE. g-1) based on the formula described by Gouveia and co-authors [30] i.e.

$$\begin{aligned}&\text{Total flavonoid content in mg/g}\\&\quad= \text{Concentration of catechin established from the curve in mg/ml)}\\&\qquad\times \text{Volume of the extract solution in mL/ weight of the extract in g.}\end{aligned}$$

Quantitative determination of tannins

The method of Amadi et al. was used [32] and Ejikeme et al. [33] were used. Briefly, Sodium tungstate (Na_2WO_4); 50 g was dissolved in distilled water (37 mL) to make the Folin-Denis reagent. Phosphomolybdic acid ($H_3PMo1_2O_40$); 10 g and Orthophosphoric acid (H_3PO_4); 25 mL were then added. The mixture was refluxed for 2 h, allowed to cool before distilled water was added. Tannic acid (0.2 g) was dissolved in distilled water, transferred to a 200 mL volumetric flask, and made up to the mark to make a 1 mg/mL stock solution. Serial dilutions of the tannic acid solution ranging from 0.2–1.0 mg/mL were pipetted into 25 mL volumetric flasks. Folin-Denis reagent (1.25 mL) was added to each flask followed by Na_2CO_3 solution (2.5 mL). The mixture was made to 100 mL with distilled water and allowed to stand for ½ and hour in a water bath at 25 °C. Absorbance was determined at 700 nm and the calibration curve was plotted. Each extract was weighed (0.5 g), transferred to a conical flask, and dissolved in 50 mL of distilled water. The

mixture was boiled gently for 60 min on an electric hot plate and filtered using into a 50 mL volumetric flask. Folin-Denis reagent (2.5 mL) and saturated Na_2CO_3 solution (5 mL) was added to 25 mL of distilled water and 5 mL of diluted extract. The solution was thoroughly agitated before being allowed to stand for ½ an hour in a 25 °C water bath. Absorbance was read at 700 nm and the tannic acid content of the extracts was calculated using the formula described by Sheikh and colleagues [34]. i.e.

$$\text{Tannic acid (mg/100 g)} = \frac{C \times \text{extract volume} \times 100}{\text{Aliquot volume} \times \text{weight of sample}} \quad [34]$$

where C is the concentration of tannic acid read off the graph.

Antimicrobial activity assay

Preparation of bacterial and fungal suspension

The method of Teh et al. was used with minor modifications [35]. Briefly, bacterial stock cultures were sub-cultured on blood agar and incubated at 37 °C for 24 h. Fungal stock cultures were sub-cultured in Sabouraud dextrose broth at 35 °C for 48 h. The test strains were suspended in sterile saline to give a final density of 1.5×10^6 CFU/mL of bacteria or 1.5×10^5 spores/mL of fungi.

Determination of the minimum inhibitory concentration (MIC) of extracts

The extracts were tested against bacterial (*Escherichia coli* ATCC 25922*, Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778) and fungal species (*Candida albicans* ATCC 10231) using broth dilution method as adopted from the method of Teke et al. with slight modifications [36]. About 1.6 g of the crude extracts were transferred into 4 mL of pre-sterilized Muller Hinton Broth and Sabouraud dextrose broth for bacteria and fungi respectively contained in a sterilized 10 mL test tube. Each test tube was clearly labeled and put in a test tube rack. Serial two-fold dilutions of plant extracts of 400, 200, 100, 50, 25 and 12.5 mg/mL concentration were prepared. Using a sterile 1 mL pipette, 0.1 mL of bacterial and fungal suspension was dispensed into each of the test tubes. A test tube containing Muller Hinton Broth without extract and 0.1 mL of the inoculum was used as a negative control. The tubes were incubated at 37 °C for 24 h for bacteria and 35 °C for 48 h for fungi. All experiments were carried out in triplicate. A two-fold dilution of conventionally used antimicrobials (Amoxicillin, Gentamicin, and Nystatin) were used as positive controls for gram-positive bacteria, gram-negative bacteria, and fungi respectively. Visual turbidity after incubation was used for inference. The minimum inhibitory concentration of the extracts and controls was determined from readings on culture plates and was defined as the lowest concentration of these substances that completely inhibited the growth of the different organisms [37].

Determination of the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of extracts

The method of Mostafa et al. [21] was used to determine the minimum bactericidal concentration (MBC) of the extracts and controls. The method of Wayne et al. [38] was used to determine the minimum fungicidal concentration (MFC) of the extracts and controls. In the determination of the MBC of the extracts, a sterile pipette was used to draw 0.1 mL suspension from the two lowest concentrations of extracts that exhibited invisible growth in the MIC test tubes. These were then sub-cultured onto sterile Muller Hinton Agar plates, incubated at 37 °C for 24 h and microbial growth was examined for each plant extract at different test concentrations. The MBC of the test samples (extracts and controls) was taken as the concentration that did not exhibit any bacterial growth on freshly inoculated agar plates. On the other hand, the MFC was defined as the lowest concentration of test substance (extracts and controls) that prevented visible growth of the fungus.

Artemia salina (Brine shrimp) lethality assay

The method of Meyer et al. was used [39]. Brine shrimp (*Artemia salina*) eggs were hatched at room temperature in a shallow rectangular dish containing the marine salt solution prepared by dissolving 38.5 g of commercial sea salt in 1 L of distilled water [39]. Ten, 48-h brine shrimp larvae were transferred from the hatching trough to 5 mL sample vials. Aliquots (5, 50, and 500 µl) of 5 mg/ml stock solutions of the extracts and standard (vincristine sulphate) were pipetted into the sample vials and made up to the mark with marine salt solution to a final concentration of 10,100, and 1000 µg/mL respectively [39]. Distilled water was used as a control. All experiments were carried out in quintuples and surviving larvae were counted after 24 h. Probit analysis was used to determine the median lethal concentration (LC_{50}) of the extracts and the standard cytotoxic drug [40]. Meyer's and Clarkson's toxicity indices were used to classify the acute toxicity of the extracts and standard drug [41, 42].

Results

The ethnobotanical information on the plant species used in this study and the extraction yield is summarized in Table 1. The percentage yield of the aqueous extracts was 21.20%, 17.80%, and 20.20% respectively for *Acanthus polystachyus, Keetia gueinzii,* and *Rhynchosia elegans.* Table 1. The percentage yield of the acetone extracts was 4.30%, 4.90%, and 0.80% respectively for *Acanthus polystachyus, Keetia gueinzii,* and *Rhynchosia elegans.* Table 1. The percentage yield of the methanol extracts was 20.20%, 0.80%, and 5.20% respectively for *Acanthus polystachyus, Keetia gueinzii,* and *Rhynchosia elegans.* Table 1**.**

The results of the Preliminary phytochemical screening of crude root, root bark, and root tuber extracts of *Acanthus polystachyus, Keetia gueinzii,* and *Rhynchosia elegans* are summarized in Table 2**.** The aqueous root extracts of *Acanthus polystachyus* contained glycosides, phenols, saponins, and tannins. Table 2. The acetone root extracts of *Acanthus polystachyus* contained alkaloids, flavonoids, glycosides, and phenols. Table 2. The methanol root extracts of *Acanthus polystachyus* contained alkaloids, flavonoids, glycosides, phenols, saponins, and terpenoids. Table 2. The aqueous root bark extracts of *Keetia gueinzii* contained glycosides, phenols, and tannins. Table 2. The acetone root bark extracts of *Keetia gueinzii* contained alkaloids, flavonoids, glycosides, and phenols. Table 2. The methanol root bark extracts of *Keetia gueinzii* contained alkaloids, phenols, saponins, and tannins. Table 2. The aqueous root tuber extracts of *Rhynchosia elegans* contained phenols, saponins, tannins, and terpenoids. Table 2. The acetone root tuber extracts of *Rhynchosia elegans* contained alkaloids, glycosides, flavonoids, phenols, and tannins. Table 2. The methanol root tuber extracts of *Rhynchosia elegans* contained phenols and saponins. Table 2. The minimum inhibitory/bactericidal/fungicidal concentrations of the crude root, root bark, and root tuber extracts of *Acanthus polystachyus, Keetia gueinzii, and Rychnosia elegans* are summarized in Table 3**.**

The antimicrobial properties of the acetone extracts of *Acanthus polystachyus* and *Keetia gueinzii* against gram-positive *Bacillus cereus* were better than the activities of the aqueous and methanol extracts of the plants. Table 3**.** However, the observed antimicrobial properties were weaker than the standard drug (Amoxicillin). Table 3**.** Aqueous and acetone extracts of *Acanthus polystachyus* were ineffective against *Staphylococcus aureus* at the doses tested. Table 3. The same was true for aqueous and acetone extracts of *Rhynchosia elegans.* Table 3. The aqueous and acetone extracts of *Keetia guenzii* exhibited the best antimicrobial properties against *S. aureus.* Table 3**.** However, the observed activity was weaker than the standard drug (Amoxicillin). Table 3 All of the prepared extracts of the plants had poor efficacy against *E. coli* and *C. albicans.* Table 3**.** The brine shrimp cytotoxicity of the

Table 1 Ethnobotanical information on the plant species used in this study and their extraction yield

Species	Family	Local name	Voucher number	Part used	Extraction yield (%)		
					Aqueous	Acetone	Methanol
Acanthus polystachyus	Acanthaceae	Nyanandi	JM2019/284/003	Root	21.20	4.30	10.10
Keetia gueinzii	Rubiaceae	Atego	JM2019/264/001	Root bark	17.80	4.90	10.90
Rhynchosia elegans	Leguminosae	Jandalusi/Jandarusi	JM2019/284/002	Root tuber	20.20	0.80	5.20

Table 2 Preliminary phytochemical screening of crude root, root bark, and root tuber extracts of *Acanthus polystachyus*, *Keetia gueinzii*, and *Rhynchosia elegans* respectively

Plant metabolite	Tests	Root extracts of *Acanthus polystachyus*			Root bark extracts of *Keetia gueinzii*			Root tuber extracts of *Rhynchosia Elegans*		
		Aqueous	Acetone	Methanol	Aqueous	Acetone	Methanol	Aqueous	Acetone	Methanol
Alkaloids	Dragendorff's test	−	+	+	−	−	+	−	+	−
Flavonoids	Alkaline reagent test	−	+	+	+	−	−	−	−	−
Glycosides	Keller-killiani test	+	+	+	+	+	−	−	+	−
Phenols	Ferric chloride test	+	+	+	+	+	+	+	+	+
Saponins	Froth test	+	−	+	+	−	+	+	−	+
Tannins	Ferric chloride test	+	−	−	+	+	+	+	+	−
Terpenoids	Salkowski test	−	−	+	−	−	−	+	−	−

+: metabolite present; −: metabolite absent

Table 3 The minimum inhibitory/bactericidal/fungicidal concentrations of crude root, root bark, and root tubr extracts of *Acanthus polystachyus, Keetia gueinzii, and Rychnosia elegans* respectively

Type of pathogen	Plant extract	MIC (mg/mL)	MBC (mg/mL)	MFC (mg/mL)	MBC/MIC	MFC/MIC
Bacillus cereus (Gram + ve)	*Acanthus polystachyus*					
	Aqueous	25	50	–	2	–
	Acetone	12.5	12.5	–	1	–
	Methanol	100	200	–	2	–
	Keetia gueinzii					
	Aqueous	50	100	–	2	–
	Acetone	12.5	12.5	–	1	–
	Methanol	25	50	–	2	–
	Rychnosia elegans					
	Aqueous	ND	ND	–	ND	–
	Acetone	*	*	–	*	–
	Methanol	ND	ND	–	ND	–
Staphylococcus aureus (Gram + ve)	*Acanthus polystachyus*					
	Aqueous	ND	ND	–	ND	–
	Acetone	ND	ND	–	ND	–
	Methanol	200	400	–	2	–
	Keetia gueinzii					
	Aqueous	12.5	12.5	–	1	–
	Acetone	12.5	12.5	–	1	–
	Methanol	25	50	–	2	–
	Rychnosia elegans					–
	Aqueous	ND	ND	–	ND	–
	Acetone	*	*	–	*	
	Methanol	200	400	–	2	
	Amoxicillin	0.00156	0.00156	–	1	–
Escherichia coli (Gram –ve)	*Acanthus polystachyus*					
	Aqueous	ND	ND	–	ND	–
	Acetone	ND	ND	–	ND	–
	Methanol	200	400	–	2	–
	Keetia gueinzii					
	Aqueous	200	400	–	2	–
	Acetone	200	400	–	2	–
	Methanol	200	400	–	2	–
	Rychnosia elegans					
	Aqueous	ND	ND	–	ND	–
	Acetone	*	*	–	*	–
	Methanol	ND	ND	–	ND	–
	Gentamycin	0.0156	0.0156	–	1	–
Candida albicans	*Acanthus polystachyus*					
	Aqueous	ND	–	ND	–	ND
	Acetone	400	–	400	–	1
	Methanol	200	–	400	–	2
	Keetia gueinzii					
	Aqueous	ND	–	ND	–	ND
	Acetone	200	–	400	–	2
	Methanol	100	–	200	–	2
	Rychnosia elegans					
	Aqueous	ND	–	ND	–	ND

Table 3 (continued)

Type of pathogen	Plant extract	MIC (mg/mL)	MBC (mg/mL)	MFC (mg/mL)	MBC/MIC	MFC/MIC
	Acetone	*	–	*	–	*
	Methanol	ND	–	ND	–	ND
	Nystatin	0.00156	–	0.00156	–	1

MIC minimum inhibitory concentration, *MBC* minimum bactericidal concentration, *MFC* minimum fungicidal concentration, *ND* not determined (did not display activity at the tested concentrations)

*Extract not subjected to antimicrobial screening

Table 4 Brine shrimp cytotoxicity of crude root, root bark, and root tuber extracts of *Acanthus polystachyus, Keetia gueinzii,* and *Rhynchosia elegans*

Plant species	Part used	Solvent used	Mortality per test dose			LC_{50} (μg/ml)	Toxicity	
			10 μg/ml	100 μg/ml	1000 μg/ml		Meyer's toxicity index [41]	Clarkson's toxicity index [44]
A. polystachyus	Roots	Acetone	0	1	50	195.17 (109.11–349.08)	Toxic	Moderately toxic
		Distilled water	0	0	0	No death	Non-toxic	Non-toxic
		Methanol	0	6	49	174.26 (93.37–325.23)	Toxic	Moderately toxic
K. gueinzii	Root bark	Acetone	0	0	0	No death	Non-toxic	Non-toxic
		Distilled water	0	0	3	148,735,210.7 (2,807,216.20–7,880,462,816)	Non-toxic	Non-toxic
		Methanol	0	0	1	1.0×10^{16} (2.0×10^{12}–5.1×10^{19})	Non-toxic	Non-toxic
R. elegans	Root tuber	Acetone	0	2	50	175.77 (98.27–314.39)	Toxic	Moderately toxic
		Distilled water	0	0	45	422.09 (199.52–893.59)	Toxic	Moderately toxic
		Methanol	0	5	50	168.76 (94.35–301.85)	Toxic	Moderately toxic
1% DMSO	–	–	0	0	0	No death	Non-toxic	Non-toxic
Vincristine sulphate	–	–	8	31	50	45.77 (19.75–106.06)	Toxic	Highly toxic

Table 5 Quantitative estimation of phytochemicals responsible for the antimicrobial properties of aqueous, acetone, and methanol extracts of *A. polystachyus, K. gueinzii,* and *R. elegans*

Plant	Extract	TFC (mg/g CE)	TPC (mg/g GAE)	TC (mg/g TAE)
A. polystachyus	Aqueous	0.32	2.14	16.61
	Acetone	15.45	8.83	8.50
	Methanol	2.62	2.22	7.66
K. gueizii	Aqueous	16.32	3.00	80.65
	Acetone	15.45	3.64	87.71
	Methanol	18.63	3.54	85.20
R. elegans	Aqueous	2.37	2.14	9.27
	Acetone	7.07	3.28	25.63
	Methanol	3.67	2.36	17.31

TFC total flavonoid content, *mg* milligrams, *g* grams, *GAE* gallic acid equivalents, *TPC* total phenolic content, *CE* catechin equivalents, *TC* tannin content, *TAE* tannic acid equivalents

crude root, root bark, and root tuber extracts of *Acanthus polystachyus, Keetia gueinzii,* and *Rhynchosia elegans* is summarized in Table 4. The acetone and methanol extracts of *Acanthus polystachyus* roots and *Rhynchosia elegans* root tubers were cytotoxic in brine shrimp with LC_{50} values less than 100 μg/mL. Table 4**.** The aqueous

extracts of the root bark of *Acanthus polystachyus,* the aqueous, acetone, and methanol extracts of the root bark of *Keetia gueinzii*, and the aqueous extracts of the root tubers of *Rhynchosia elegans* were non-cytotoxic to brine shrimp with LC_{50} values of less than 100 µg/mL. Table 4**.**

The results of the quantitative analysis of phytochemicals present in the aqueous, acetone, and methanol extracts of the root, root bark, and root tuber extracts of *Acanthus polystachyus, Keetia gueinzii,* and *Rhynchosia elegans* are summarized in Table 5*.* The acetone extract of *Acanthus polystachyus* had the highest total phenolic content (8.83 mg/g GAE) while the methanol extract of *Acanthus polystachyus* and the aqueous extract of *Rhynchosia elegans* had the least total phenolic content (2.14 mg/g GAE)*.* Table 5**.** The acetone extract of *Keetia gueinzii* had the highest tannin content (87.71 mg/g TAE) while the methanol extract of *Acanthus polystachyus* had the least tannin content (7.66 mg/g TAE)*.* Table 5**.**

Discussion

Antimicrobial agents have kept disease-causing microbial infections at bay for many decades[10, 43, 44]. However, the tide seems to be changing because microbes continue to develop elaborate mechanisms which confer resistance to the antimicrobial agents which are currently available in the market [45–47]. The development of new drugs to combat the rising threat of antimicrobial resistance is impeded by reduced economic incentives, challenging regulatory requirements, and other factors [48, 49]. New policies and renewed research are therefore urgently required to mitigate the unfolding crisis of antimicrobial resistance. Medicinal plants and their extracts may be suitable alternatives in the quest to solve this crisis. Indeed, literature is rife with reports on the efficacy of medicinal plants against common bacterial and fungal pathogens [50–53]. The practice of traditional herbal medicine in Kenya is deeply entrenched in society. However, scientific data to support the claims emanating from the practice of traditional herbal medicine in the country is scanty. *Acanthus polystachyus, Keetia gueinzii,* and *Rhynchosia elegans* are routinely used in Kisumu County to manage a host of diseases including microbial infections [11]. There is a paucity of information on the phytochemical composition, antimicrobial properties, and safety of these plants. This study aimed to determine the phytochemical composition, antimicrobial properties, and safety of different solvent extracts of *Acanthus polystachyus, Keetia gueinzii,* and *Rhynchosia elegans*. The yield of the prepared extracts of *Acanthus polystachyus, Keetia gueinzii*, and *Rhynchosia elegans* ranged from 0.8% w/w to 21.2% w/w. This was higher than the extract yield of *Oxalis corniculata, Cinnamomum tamala, Ageratana adenophora*, and *Artemesia vulgaris* which ranged from 2.38% w/w to 9.35% w/w [54]. The values were similar to the 0.89% w/w to 30.27% w/w yield of water, 50% ethanol, 70% ethanol, 95% ethanol, acetone, and dichloromethane extracts of *Blepharis linarifolia, Tinospora bakis, Cyperus rotundus, Maema psudopetalosa, Dicoma tomentosa,* and *Guiera senegalensis,* [55].

The solvents which are used during the extraction period have been reported to exert some influence on the nature and the quantity of secondary metabolites [29, 56, 57]. Therefore, the choice of solvent is a key aspect of achieving the desired pharmacological activity of the plant extracts. In the present study, acetone extracts had the highest total phenolic, total flavonoid, and tannin content. A study on the comparative effects of some extraction solvents on the antimicrobial activity of *Eucalyptus camaldulensis* revealed that both the type and concentration of the solvent greatly affected the antimicrobial properties of the plant [58]. It was reported that the 30% acetone extract of *Eucalyptus camaldulensis* showed the highest inhibition of *Acinetobacter baumanni* [58]. This trend was also observed when the acetone extracts of *Acanthus polystachyus* and *Keetia gueinzii* were tested against the gram-positive *Bacillus cereus* pathogen.

The minimum inhibitory concentration (MIC) of the extracts against the gram-positive *Bacillus cereus* ranged from 12.5 to 100 mg/mL. This was higher than the MIC reported on Thai medicinal plant extracts but was lower than the MIC value reported for the acetone extract of *Nigella sativa* (black curcumin) [55, 59]. The minimum bactericidal concentration (MBC) of the extracts against gram-positive *Bacillus cereus* ranged from 12.5 to 200 mg/mL. This was higher than the 12.5–25 mg/mL range reported for the methanol extract of *Rosa damascene* [60]. The minimum inhibitory (MIC) of the extracts against gram-negative *Staphylococcus aureus* ranged from 12.5 to 200 mg/mL while the minimum bactericidal concentration (MBC) ranged from 12.5 to 400 mg/mL. These values were higher than what was reported on 239 traditional Chinese extracts reported by Kim and colleagues who reported MIC values ranging from 0.10 to 12.5 mg/mL and MBC values ranging from 0.78 to 25 mg/mL [61]. The MIC of the extracts against gram-negative *E. coli* ranged from 200 mg/mL to ineffective. This was higher than the MIC values reported for aqueous and ethanol extracts of the fruit, leaves, and stem of *Solanum incanum* reported by Sbhatu and Ahraha [62]. The MBC of the extracts against gram-negative *E. coli* ranged from 400 to ineffective. These values were comparable to the values reported for extracts prepared from *Psidium guajava, Salvia officinalis, Ziziphus sphinachristii, Morus alba,* and *Olea Europea* [63]. The MIC of the extracts against *Candida albicans* ranged from 100 mg/

mL to ineffective. These values were higher than what was reported on essential oils from *Nigella sativa, Syzgum animaticum-Thymus vulgaris* mixture, and *Origanum vulgare* which ranged from 0.3 to 48.8 mg/mL [64]. The solvent extracts studied had MIC or MBC values that were up to 800 fold less effective than the standard drugs. However, it may be of some interest that the extracts of the root bark of *Keetia gueinzii* generally exhibited a broad spectrum of activity against gram-positive (*Bacillus cereus* and *Staphylococcus aureus*), gram-negative (*Escherichia coli*) bacteria and fungal (*Candida albicans*) microorganisms. The choice of the solvent appeared to affect the antimicrobial activity of root barks of *Keetia gueinzii.* Extracts prepared by using highly polar solvents such as water and methanol displayed better antimicrobial activity against *Bacillus cereus* than the extract prepared using acetone. It is worth noting that phenols, saponins, and tannins were common compounds in both the aqueous and methanol extracts but were absent in the acetone extracts. It could be argued that these compounds may have some role in the antimicrobial activity exhibited by these extracts. Moreover, the presence of alkaloids in the methanol extract of *Keetia gueinzii* may be the reason for the good antimicrobial activity of this extract against *Staphylococcus aureus*. Phenols have been shown to significantly reduce biofilm mass formed by *C. albicans* thereby reducing the number of viable cells [65]. This may explain the activity of the aqueous and methanol extracts of *Keetia gueinzii* against *C.albicans.*

The total flavonoid content (TFC) of the extracts ranged from 0.32 to 18.63 mg/g catechin equivalents (CE) while the total phenolic content (TPC) ranged from 2.14 to 8.83 mg/g gallic acid equivalents (GAE). These values were lower than the TPC and TFC of *Alternanthera sesailis, Basella alba, Cassia tora, Digera muricata, ipomoea aquatica, Leucas cephalotes, Portulacea oleracea,* and *Solanum nigrum* [66]. The tannin content of the extracts ranged from 7.66 to 87.7 mg/g TAE. These values were lower than the aqueous extracts of *Cassia asiatica* and *Musa acuminata* [67].

The brine shrimp cytotoxicity of the extracts ranged from 168.76 to 148, 735, 210.7 μg/mL. These values were comparable to those reported by Tlili and colleagues on *Hernaria fontanessi, Ziziphus lotus, Plantago ovata,* and *Thymelaeae hirsuta* [68]. Based on the brine shrimp lethality model, acetone and methanol extracts of *Acanthus polystachyus* and all extracts of *Rhynchosia elegans* were toxic in the brine shrimp model. The presence of alkaloids and flavonoids in the acetone and methanol extracts of *Acanthus polystachyus* could be the reason for the observed toxicity. A study on the brine shrimp cytotoxicity of endemic Papuan plants e.g. *Piper methysticum* and *Evodia suaveolens* were not only cytotoxic to brine shrimp but were also rich in alkaloids and flavonoids [69]. Further evidence of the brine shrimp cytotoxicity of alkaloids is exemplified in the work of Seremet and colleagues who reported that pyrrolizidine alkaloids isolated from *Senecio vernalis, Symphytum officinale, Petasites hybrid,* and *Tussilago farfara* were cytotoxic to brine shrimp [70]. Popovici and colleagues evaluated the antioxidant and *Artemia salina* cytotoxic activities of *Usnea barbata* dry extracts in different solvent systems and reported that all extracts (acetone, ethyl acetate, ethanol, methanol, and water) had high cytotoxicity in *Artemia salina* [71]. This is in agreement with some of the findings of this study particularly in the case of acetone and methanol extracts of *Rhynchosia elegans*. However, distilled water extracts of *Acanthus polystachyus*, acetone root bark extracts of *Keetia gueinzii*, and distilled water and methanol root bark extracts of *Keetia gueinzii* were relatively safe in *Artemia salina.*

Conclusions

The use of the roots, root barks, and root tubers of *Acanthus polystachyus, Keetia gueinzii,* and *Rhynchosia elegans* is limited by poor antimicrobial efficacy against *Bacillus cereus, Staphylococcus aureus, Escherichia coli*, and *Candida albicans.* Except for the root barks of *Keetia gueinzii*, the use of all other plants tested is limited by safety concerns.

Abbreviations

WHO: World Health Organization; FVM: Faculty of Veterinary Medicine; BAUEC: Biosafety animal use and ethics committee; NACOSTI: National Commission for Science, Technology, and Innovation; GAE: Gallic acid equivalents; CE: Catechin equivalents; TAE: Tannic acid equivalents; TPC: Total phenolic content; TFC: Total flavonoid content; TC: Tannin content; UV–VIS: Ultraviolet–visible; CFU: Colony-Forming Unit; MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; ATCC: American Type Culture Collection; MFC: Minimum Fungicidal Concentration; LC_{50}: Median lethal concentration in brine shrimp.

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Authors' contributions

JKM: Conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, visualization, writing original draft, and writing review and editing. JMN: Conceptualization, investigation, methodology, supervision, validation, writing review, and editing. JMM: Conceptualization, investigation, methodology, supervision, validation, writing review, and editing. MOO: Formal analysis, investigation, validation, visualization, writing original draft, writing review, and editing. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Plant authentication
Samples of the whole plant of ***Acanthus polystachyus, Keetia gueinzii,*** and ***Rhynchosia elegans*** were collected, transported to the University of Nairobi herbarium where they were authenticated by Mr. Kimeu Musembi. Voucher specimens were deposited for future reference; JM2019/284/003 for roots of ***Acanthus polystachyus***, JM2019/264/001 for root bark of ***Keetia gueinzii***, and JM2019/284/002 for root tubers of ***Rhynchosia elegans***.

Competing interests
The authors declare that they have no competing interests.

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