Research Journal of

Medicinal Plant

ISSN 1819-3455

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 6 (5): 370-382, 2012
ISSN 1819-3455 / DOI: 10.3923/rjmp.2012.370.382
© 2012 Academic Journals Inc.

Antimicrobial Activity and Safety of two Medicinal Plants Traditionally used in Bomet District of Kenya

[1,2]R.K. Korir, [2]C. Mutai, [1]C. Kiiyukia and [2]C. Bii

[1]Institute of Tropical Medicine and Infectious Diseases, P.O. Box 54840, Nairobi 00200, Kenya
[2]Kenya Medical Research Institute, P.O. Box 54840, Nairobi 00200, Kenya

Corresponding Author: Richard Kipserem Korir, Kenya Medical Research Institute, P.O. Box 54840, Nairobi, Kenya

ABSTRACT

Indigenous rural communities in the tropics manage microbial diseases using herbal concoctions. The efficacy and safety of most of the herbal preparations have not been proven scientifically. Extracts from two selected plant species were evaluated for *in vitro* antimicrobial activities and toxicity. Extraction was done using hexane, dichloromethane, methanol and water. Hexane extracts of *Senna didymobotrya* was active against *Microsporum gypseum* with 16.0 mm inhibition zone diameters and no activity against other test isolates. Dichloromethane extracts had good activities against *Trichophyton mentagrophyte* and *Microsporum gypseum* with 18.0 and 15.0 mm inhibition zones, respectively. Minimum inhibition concentration of *Cyathula polycephala* extracts against bacterial isolates ranged from 25.0 to 3.124 mg mL^{-1}. Methanol extracts of *C. polycephala* had a CC_{50} of 100.0%. The water extracts recorded CC_{50} of 23.75 and 31.56% at 1000 and 100 mg mL^{-1} concentration, respectively. The methanol extracts recorded a CC_{50} of 99.34 and 100.0% at 1000 and 100 mg mL^{-1} concentration, respectively. The DCM extract of *S. didymobotrya* was very toxic at 5000 mg kg^{-1} and killed 80% of mice. In both the cell and acute toxicity, it was observed that extracts at high concentration and at a high dose tend to be toxic. Most extracts were active against a wide range of bacteria than fungi.

Key words: Ethnobotanical, *in vitro, in vivo*, antimicrobials, toxicity, inhibition zones

INTRODUCTION

People all over the world have used plants as medicines from time immemorial. It is estimated by WHO that 80% of the population, majority of this in developing countries, still rely on plant-based medicine for primary health care (Evans, 1997). Herbal drugs are prepared from various parts of the plant such as leaves, stem, roots, seeds, tubers or exudates (Mukherjee, 2002). There are more than 1,340 plants known to be potential sources of antimicrobial compounds but few have been systematically studied scientifically (Wilkins and Board, 1989). Some plant like *Heteromorpha trifoliata* (Wendl.) Eckl. and Zeyh. is used traditionally to treat stomach problems. It has been shown to have strong activity against several species of bacteria including *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella gallinarum* and *Staphylococcus albus* (Desta, 1993; Baker *et al.*, 1995). Although, plants have been extensively used, specific evaluation on toxicity has not been done and could lead to serious complications (Rahman *et al.*, 1996).

Indiscriminate exposure to antibiotics in patients hastens bacteria mutations that bring about drug resistance. This situation produces bacteria with greater ability to survive even the strongest antibiotics (WHO, 2002). More than 70% of the bacteria causing these infections are resistant to at least one of the drugs commonly used to treat them. Persons infected with drug resistant strains are more likely to have longer hospital stay and require treatment with second or third line drugs that may be effective but more toxic and expensive (Brun-Buisson *et al.*, 1987). Drug resistant bacteria have mainly been of nosocomial origin (Brun-Buisson *et al.*, 1987).

The antimicrobial resistance problem has been worsened by HIV pandemic due to emergence of opportunistic infection thereby leading to indiscriminate use of antibiotics (WHO, 2004). The antimicrobial resistance to the available drugs is driving up healthcare costs, increasing the severity of disease, morbidity and mortality. The situation calls for urgent research on any potential source of new effective antimicrobials. Plants products are some of such sources which have not been exhaustively utilized. Many plant preparations are used by the Kipsigis community of Kenya without any available scientific study on efficacy or safety. In this study, selected medicinal plants used by the Kipsigis ethnic community of Kenya, were subjected to a systematic screening for antimicrobial activity, *in vitro* and *in vivo* toxicity.

Senna didymobotrya (Fresen) Irwin and Barneby (Caesalpinaceae) is used traditionally by the Kipsigis community for the control of Malaria as well as diarrhea. It is used to treat skin conditions in humans and widely used to treat livestock infections in Kenya as documented by Njoroge and Bussmann (2007). Antimicrobial properties of this plant from Bomet district have not been documented.

Cyathula polycephala auct. belongs to the family Amaranthaceae. It is a genus of medicinal and ornamental plants and the species are distributed in Africa, Asia, Oceania and the Americas. The leaves are burned into ashes and are used to treat diabetes among the Mbeere and Ameru communities in Kenya (Kareru, 2007). Brine shrimp toxicity as well as antiplasmodial properties of methanol extracts of *Cyathula polycephala* has been documented by Wanyoike *et al.* (2004). The leaves are burned and the ashes are applied to skin infection while the roots are boiled and used to treat pneumonia by the Kipsigis community. The antimicrobial properties of this plant have not been documented.

MATERIALS AND METHODS

Collection of the plants from the field: The *Senna didymobotrya* and *Cyathula polycephala* plants were collected from Bomet District. Selection of the plants was based on available ethnobotanical information from traditional health practitioners consulted during the pilot study as well as available literature. The plants are used to treat skin conditions and stomach problem. The herbalist advised on the samples to be collected and the plant part used for different kinds of ailments. The plants parts commonly used and collected were from the stem barks. The study plant materials were photographed and collected for authentification at the East African Herbarium (Table 1). The samples for extractions were collected in paper bags and delivered to the Medicinal Chemistry Laboratory of the Kenya Medical Research Institute (KEMRI), Centre for Traditional Medicine and Drug Research for processing.

Extraction of plant materials: Plants materials were dried at room temperature, grounded into a fine powder and stored in a cool and dry place. Total extraction of each plant material was prepared by mixing with solvent in the ratio of 1:10 (plant material/solvents). This procedure was

Table 1: Plants collected for the study

Botanical name/part collected	Family name	Vernacular name (Kipsigis)	Herbarium No.
Senna didymobotrya-stem bark	Caesalpinaceae	Senetwet	KIFS 038
Cyathula polycephala-stem bark	Amaranthaceae	Mangoita	KIFS 092

KIFS is the herbarium number assigned to the plants

Table 2: Thin layer chromatography visualization reagents

Compounds	Visualizing reagents
Terpenoids	Anisaldehyde
Alkaloids	Dragendorff reagents
Flavonoids	Ammonia fumes
Phenolics	Ethanolic potassium hydroxide
Anthraquinones	Methanolic potassium hydroxide (Kedde reagents)

done successively using hexane, dichloromethane and methanol where, the plant materials were soaked in 1000 mL conical flasks for 12 h. The extracts were filtered using Whatman No. 1 filter paper and solvents removed through evaporation under reduced pressure at 45°C. The extracts were kept in stoppered sample vials at 4°C until they were used (Stein *et al*., 2005; Chhabra *et al*., 1984). Total water extract of each plant material was done by soaking a weighed amount of the dry powder (27-50 g) in distilled water and shaken for two hours with an electric shaker at 65°C. The suspension was filtered and the filtrate was kept in a deep freezer before it was evaporated to dryness by freeze drying. The lyophilized dry powder was collected in stoppered sample vials, weighed and kept in a desiccator, to avoid absorption of water, until they were used. Table 1 and 2 presented the plants and reagents collected for study.

Test strains: Test strains were chosen based on the ethnobotanical exploitation of the plants. The following are the standard organisms and clinical isolates used in this study.

Bacterial isolates:

- **Gram positive:** *Staphylococcus aureus* ATCC 25923 and Methicillin resistant *Staphylococcus aureus* (MRSA) clinical isolate
- **Gram negative:** *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae*. (Clinical isolate)
- **Fungi isolates:** Yeast; *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 90019, *Candida krusei* ATCC 5862 and *Cryptococcus neoformans* ATCC 66031
- Dermatophytes; (Clinical isolates); *Microsporum gypseum* and *Trychophyton mentagrophytes*

Preparation of test organisms: Stocked bacterial strains were sub-cultured on Muller Hinton agar No. CM0337. (Oxoid Ltd, Basingstoke, Hampshire, England). Incubation was done at 37°C for 12-18 h to obtain freshly growing strains. Yeast and molds were subcultured onto Sabouraud Dextrose Agar No. CM 004 (Oxoid Ltd, Basingstoke, Hampshire, England). Each media was prepared according to the manufacturers instructions. Yeasts were incubated for 24 h while molds were incubated for 72 h at 30°C to obtain freshly growing culture (Rajakaruna *et al*., 2002).

Preparation of McFarland standard: Exactly 0.5 McFarland equivalent turbidity standards was prepared by adding 0.6 mL of 1% barium chloride solution ($BaCl_2.2H_2O$) to 99.4 mL of 1% sulphuric

acid solution (H_2SO_4) and mixed thoroughly. A small volume of the turbid solution was transferred to cap tube of the same type that was used to prepare the test and control innocula. It was then stored in the dark at room temperature (25°C). Exactly 0.5 McFarland gives an equivalent approximate density of bacteria 1×10^{-6} Colony Forming Units (CFU) mL^{-1} (Stein *et al*., 2005).

Antimicrobial screening test: From an overnight growth of the test organism, 4-6 colonies were emulsified and the suspension was adjusted to match the 0.5 McFarland's standard. Respective plates were inoculated using a sterile cotton wool swab. Antimicrobial susceptibility test was done using disk diffusion methods. Briefly, 100 mg of each extract was dissolved in 1 mL of the appropriate solvents and 10 µL of the mixture was impregnated onto 6 mm sterile filter paper disk and air dried. The disk was placed aseptically onto the inoculated plates. The bacterial and yeast cultures were incubated at 37°C for 24 and 48 h, respectively. Dermatophytes (molds) cultures were incubated at 25°C for 72 h after which the inhibition zone diameter was measured in millimeters and recorded against the corresponding concentrations as described by Elgayyar *et al*. (2001). Positive controls were set against standard antibiotics gentamicine 30 µg mL^{-1} and antifungal drugs fluconazole 30 µg mL^{-1} while negative controls were set using disk impregnated with extraction solvents.

Determination of minimum inhibitory concentration: Broth micro dilution method was used to determine minimum inhibitory concentration for the active crude extracts against the test microorganisms. The procedures were done as recommended by the National Committee for Clinical Laboratory Standards now Clinical Laboratory Standard Institute (CLSI) (Ferraro, 2003). The tests were performed in 96 well-micro-titer plates. Plants extracts dissolved in respective solvents were transferred into micro-titer plates to make serial dilutions ranging from 10^1, 10^2, 10^3........10^{10}. The final volume in each well was 100 µL. The wells were inoculated with 5 µL of microbial suspension. The yeast and bacteria were incubated at 37°C for 24 h while molds were incubated at 25°C for 3-7 days in ambient air. The MIC was recorded as the lowest extract concentration demonstrating no visible growth as compared to the control broth turbidity (Michael *et al*., 2003). Wells that were not inoculated were set to act as control. All the experiments were done in triplicates and average results were recorded.

Cell toxicity: The cytotoxic concentration causing 50% cell lysis and death (CC_{50}) was determined for the extracts by a method described by Kurokawa *et al*. (2001). The extracts of the active plants were tested for *in vitro* cytotoxicity, using Human Embryonic Lung Fibroblast (HELF) Vero-199 cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Mosmann, 1983). The HELF cells were cultured and maintained in Minimum Essential Medium (MEM) supplemented with 10% Featal Bovin Serum (FBS). The cells were cultured at 37°C in 5% CO_2, harvested by trypsinization, pooled in a 50 mL vial. Approximately 100 µL cell suspension (1×10^5 cells mL^{-1}) added to each well in a 96-well micro-titer plate. Each sample was replicated 3 times and the cells incubated at 37° in 5% CO_2 for 24 h for attachment. 150 µL of the highest concentration of each of the test samples (a serial dilution, prepared in MEM) added into the same row and a serial dilution done. The experimental plates were incubated further at 37°C for 48 h. The cells in media without drugs were used as controls. After 48 h of incubation, MTT (10 µL) was added into each well. The cells were incubated for 4 h or until a purple precipitate was clearly visible under a microscope. The medium together with MTT was aspirated off from the wells and

dimethlysulfoxide (DMSO) (100 µL) was added and the plates shaken for 5 min. The absorbance for each well was measured at 562 nm in a micro-titre plate reader (Mosmann, 1983) and percentage Cell Viability (CV) calculated via an excel program with the formula:

$$CV(\%) = \frac{\text{Average abs of duplicate drug wells-Average abs of blank wells}}{\text{Average abs of control wells}} \times 100$$

Determination of acute toxicity: Fifteen Swiss albino mice used in the study were sourced from KEMRI animal house facility. Healthy mice (weight 20-22 g) were divided into two groups (control and treatment group) each cage with five mice. The mice were allowed to have access to water and food, except for a short fasting period of 12 h before oral administration of single dose of the test sample. The active extracts suspension was administered orally at logarithmic dose ranging between 1000-5000 mg kg^{-1} b.wt. In this study, two dose levels of 1000.0 and 5000.0 mg kg^{-1} body weight were used. The general behavior of mice was observed continuously for 1 h after the treatment and then intermittently for 4 h and thereafter over a period of 24 h (Twaij *et al*., 1983). The mice were observed further for up to 14 days following treatment for any sign of restlessness and the latency of death. The LD_{50} value was determined according to a method described by Thompson (1985). During experiment all the dead mice were disposed according to KEMRI biosafety guidelines. After the experiment, all the mice were sacrificed using chloroform and the carcasses incinerated.

Phytochemical evaluation: Phytochemical screening was done on the active extracts to identify the active chemical principles. Based on the initial bioassays the plant extracts exhibiting biological activity were screened for groups of chemical constituents. Thin Layers Chromatography (TLC) plates were developed with Chloroforms: Methanol (98:2) with five drops of glacial acetic acid before spraying with thin layer chromatography visualization reagents giving specific reactions (Harborne, 1998).

Statistical data analysis: The results were subjected to statistical analysis for qualification of variability. Statistical Packages for Social Scientist (SPSS) Version 12.0 was utilized which enabled the Analysis of Variance by one way (ANOVA) to establish the significance variability between and within groups (Plants, Solvents and organisms). Bioactivity was used as an independent variable to establish significance p value of 0.05 at 95% confidence interval. The cytotoxic concentration causing 50% cell lysis and death (CC_{50}) was determined for the extracts. Three experiments were performed for each of the *in vivo* study. *In vivo* toxicity was checked for significant differences in lethal dose resulting to fifty percent deaths (LD_{50}) values between the tests and the controls groups.

RESULTS

Yields for sequential extractions: The percentage yields of *Senna didymobotrya* Hexane bark extract was 0.166% while *Cyathula polycephala* was 0.987%. Water extracts produced 2.836 and 7.023% yields for *Senna didymobotrya* and *Cyathula polycephala*, respectively. The results are as shown in Table 3.

Bioactivities of the plants extracts: The methanol and dichloromethane extracts of *Senna didymobotrya* had the highest activity compared to water and hexane. Hexane extracts had no activity against *S. aureus,* MRS. *aureus, Escherichia coli* and *Klebssiela pneumoniae* and the water

Table 3: Percentage yields for sequential extractions

Plant	Solvent	Part extracted (g)	Initial weights (g)	Extract weight in (g)	Yield (%)
Senna didymobotrya	Hexane	Bark	100.0	0.166	0.166
	DCM	Bark	100.0	1.372	1.372
	Methanol	Bark	100.0	1.093	1.093
	Water	Bark	27.0	2.836	2.836
Cyathula polycephala	Hexane	Bark	30.0	0.296	0.987
	DCM	Bark	30.0	0.104	0.347
	Methanol	Bark	30.0	1.792	5.973
	Water	Bark	26.0	1.826	7.023

Percentage yields for sequential extractions (% yields) = extracted weights/initial weights×100 (All weights in grams)

Table 4: Antibacterial activities *Senna didymobotrya* and *Cyathula polycephala*

Plant	Test samples (extracts/drug)	Conc. ($mg\ mL^{-1}$)	Inhibition zone diameters in millimeter for each test organisms and MIC ($mg\ mL^{-1}$)				
			S. aureus	MRSA	*P. ae*	*E. coli*	*K. pne*
Senna didymobotrya	Hexane	100	6.0-	6.0-	10.0 (100)	6.0-	6.0-
	DCM	100	9.0-	10.0 (100.0)	16.0 (12.5)	9.0-	12.0 (50.0)
	Methanol	100	19.0 (<0.1953)	11.0 (100.0)	8.0-	9.0-	12.0 (50)
	Water	100	6.0-	6.0-	10.0 (100)	6.0-	11.0 (100)
Cyathula polycephala	Hexane	100	6.0-	6.0-	7.0-	6.0-	6.0-
	DCM	100	6.0-	6.0-	7.0-	6.0-	6.0-
	Methanol	100	14.0 (6.25)	16.0 (3.12)	10.0 (100.0)	6.0-	6.0-
	Water	100	12.0 (12.5)	9.0-	11.0 (25.0)	6.0-	9.0-
-Control	DMSO	100	6.0-	6.0-	6.0-	6.0-	6.0-
+Control	Gentamicin	*30	19.0 (0.5)	9.0-	21.0 (0.5)	19.0 (0.5)	17.0 (0.5)
-Control	Water	100	6.0-	6.0-	6.0-	6.0-	6.0-

MRSA: Methicillin *Staphylococcus aureus*, *P. ae*: *Pseudomonas aeruginosa*, *K. pne*: *Klebsiella pneumonia*, Gentamicin *30 $\mu g\ mL^{-1}$, Values in brackets indicate MIC ($mg\ mL^{-1}$)

extract had activity of 10.0 mm against *Pseudomonas aeruginosa* and 11.0 mm against *K. Pneumoniae*, respectively. Methanol extract recorded relatively a moderate to low minimum inhibition concentration. The plant extracts had different MIC range for different solvents against different fungal isolates. The results are as shown in Table 4 and 5, respectively.

The methanol extract of *Cyathula polycephala* was active against methicillin resistant *Staphylococcus aureus*, *S. aureus* and *P. aeruginosa* with inhibition zone diameters of 16.0, 14.0 and 10.0 mm, respectively. The MIC for the plant in different solvents against different bacterial isolates ranged from 100.0 to 3.12 $mg\ mL^{-1}$. Methanol extract of *C. polycephala* recorded the lowest MIC of 3.12 $mg\ mL^{-1}$ against MRSA and 6.25 $mg\ mL^{-1}$ against *S. aureus*. Water extract showed less activity against *Microsporum gypseum* with 7.0 mm zone of inhibition. The plants extracts had good activities against dermatophytes while yeasts isolates recorded poor activity judged by the size of zones of inhibition. The results are as shown in Table 4 and 5, respectively.

Phytochemical screening: The plants were screened using thin layer chromatography visualization reagents. The plants extracts were found to show a positive test for the presence of phenols. Terpenoids and anthraquinones were present on *Senna didymobotrya* dichloromethane extract and absent in the other plants extracts screened. The results are as shown in Table 6.

Table 5: Antifungal activities of *Senna didymobotrya* and *Cyathula polycephala*

			Inhibition zone diameters in millimeters of each tested isolate and MIC (mg mL^{-1})					
Plant	Test samples (extracts/drug)	Conc. (mg mL^{-1})	CRPT	CA	CP	CK	TM	MG
Senna didymobotrya	Hexane	100	6.0-	6.0-	6.0-	6.0-	6.0-	16.0 (12.5)
	DCM	100	6.0-	7.0-	7.5-	7.0-	18.0 (12.5)	15.0 (25.0)
	Methanol	100	6.0-	9.0-	8.0-	7.0-	17.0 (12.5)	6.0-
	Water	100	6.0-	10.0-	7.0-	6.0-	6.0-	6.0-
Cyathula polycephala	Hexane	100	6.0-	6.0-	6.0-	6.0-	6.0-	6.0-
	DCM	100	6.0-	6.0-	6.0-	6.0-	6.0-	6.0-
	Methanol	100	6.0-	6.0-	6.0-	6.0-	14.0 (50.0)	15.0 (25.0)
	Water	100	6.0-	6.0-	6.0-	6.0-	6.0-	7.0-
-Control	DMSO	100	6.0-	6.0-	6.0-	6.0-	6.0-	6.0-
+Control	Fluconazole	*30 (0.5)	23.0 (0.5)	27.0 (0.5)	24.0 (0.5)	26.0 (0.5)	20.0 (1.0)	20.0 (1.0)
-Control	Water	100	6.0-	6.0-	6.0-	6.0-	6.0-	6.0-

CRPT: *Cryptococcus neoformans*; CA: *Candida albicans*; CP: *Candida parapsilosis*; CK: *Candida krusei*; TM: *Trichophyton mentagrophyte*; MG: *Microsporum gypseum*; Fluconazole *30 μg mL^{-1}; Values in brackets indicates MIC (mg mL^{-1})

Table 6: Phytochemical profile of the plants extracts

		Plants compounds				
Plant species	Solvent	Terpenoids	Alkaloids	Flavonoids	Phenolics	Anthraquinones
C. polycephala	M	_	_	_	+	_
S. didymobotrya	M	_	+	+	+	_
	D	+	+	+	+	+

D: Dichloromethane; M: Methanol; B: Bark; +: Present; -: Absent

Table 7: *In vitro* toxicity test results of the test extracts

		CC_{50} results in two concentrations (mg mL^{-1})	
Plant species	Extract and controls	1000.0	100.0
C. polycephala	Methanol	100.00	100.00
	Water	23.75	31.56
S. didymobotrya	DCM	11.28	28.57
	Methanol	99.34	100.00
	Water	100.00	100.00
Controls (negative and positive)	CH	100.00	100.00
	DMSO	100.00	100.00
	QC	25.28	51.94
	22651/1	7.89	11.28

Chloroquine (CQ) and TDR (22651/1 and 20186/1) drugs with an initial concentration of 100 μg mL^{-1} DMSO (Dimethylsulfoxide) were used as the control standards for the experiment

Cytotoxicity: *Cyathula polycephala* methanol extracts was very safe with a CC_{50} of 100% while water extracts were toxic with CC_{50} of 23.75 and 31.56%. The two extracts *C. polycephala* water and *S. didymobotrya* DCM were very toxic as compared to the positive control Chloroquine with CC_{50} of 25.28 and 51.94% at concentration 1000 and 100 mg mL^{-1}, respectively. The results are as shown in Table 7.

Table 8: Acute toxicity test of *Senna didymobotrya* and *Cyathula polycephala* extracts

			Mortality within 24 h			
			LD_{50} at 5000 mg kg^{-1}		LD_{50} at 1000 mg kg^{-1}	
Test extract	Weight of mice (g)		Deaths	Deaths (%)	Deaths	Deaths (%)
S. didymobotrya (DCM)	17.8±0.837	21	4/5	80	2/5	40
C. polycephala (Water)	18.0±0.707	23.8±1.304	0/5	0	0/5	0
Control	18.8±1.304	24±1.414	0/5	0	0/5	0

DCM: Dichloromethane

Acute toxicity: *Senna didymobotrya* DCM extract killed 80% of mice at a dose of 5000 mg kg^{-1} and 40% at a dose of 1000 mg kg^{-1} therefore, it LD_{50} is between 5000 and 1000 mg kg^{-1}. This suggests that the *Cyathula polycephala* extract could be safe as an antimicrobial agent at both doses although this was done on an animal model but not human beings. Other indicators of toxicity were restlessness, general body weakness, loss of appetite and fever. The results are as shown in Table 8.

DISCUSSION

Yields for sequential extractions: Water extraction produced the highest yields of as compared to all the solvents used. Methanol extracts produced higher yields compared to the other organic solvents used. In general, the percentage yields for water extracts were more than the percentage yields for organic solvents. This is due to the fact that water is very polar than organic solvents hence it is able to extract more compounds from a plant material. Kigondu (2007) also found that water extracts produced the highest yields as compared to organic solvents.

Bioactivities of the plants extracts: The results obtained in this study indicate a considerable difference in antimicrobial activity of different extracts from the two plant species. *Pseudomonas aeruginosa* was the most susceptible bacterium of all the bacterial test strains. Similarly, *T. mentagrophyte* was found to be the most sensitive fungus of all the tested fungal strains. From all the fungal strains included in the test, *C. albicans*, *C. parapsilosis, C. krusei, C. neoformans* were found to be virtually resistant to all plant extracts. *Cryptococcus neoformans* was found to be the least susceptible fungus probably because of the presence of a mucopolysacharrid capsule. The polysaccharide capsuler material in some of the pathogenic microorganism is responsible for virulence and antimicrobial resistance (Hooper, 2001). Some crude extracts were more active on fungal strains as compared to bacteria while a wide range of the extracts were moderately active against a wide range of bacteria. The least activity or no activity was evidence in several extracts with inhibition zone diameters being below 10 and 6 mm indicating no activity. In terms of inhibition zone diameters most of the extracts displays antibacterial activity amounting to 10.0 mm this can be considered moderately active in consideration that the highest is at 16.0 and the lowest is 7.0 mm. These findings are in line with the investigation of Bii *et al.* (2010) which indicates that the lowest activity was at 7.0 mm and the highest was at 18 mm in diameters. The solvents used for extraction were used as negative control while fluconazoles and gentamycin were used as positive controls. The standards drugs (gentamycin and fluconazole) inhibition zone diameters was between 9 and 21 mm which compared well with that of the plant extracts. When the antimicrobial activities of these plants extracts were

compared to that of the positive controls, only *S. didymobotrya* dichloromethane extract at a concentration of 100 mg mL^{-1} was found to have almost comparable activity to the standard gentamycin against *P. aeruginosa*. Similarly, *S. didymobotrya* and *C. polycephala* at 100 mg mL^{-1} showed comparable activity against the fungi *T. menthagrophytes* and *Microsporum gypseum* to that of fluconazole.

In general, among the tested microbial strains, bacteria were found to be more sensitive to many of the test agents than fungi. The antibacterial activity was more pronounced on the gram-negative bacteria (*K. pneumonia*) than the gram-positive bacteria (*S. aureus* and *MRSA*). The reasons for variant activities between gram negative and gram positive bacteria could be due to their morphological difference. Gram negative bacteria have an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall more permeable to lipophilic solutes while porins constitute a selective barrier to the hydrophilic. Gram positive bacterial should be more susceptible since they have only an outer peptidoclycans layer which is not an effective permeabihty barrier as reported by Nostro *et al.* (2000). But in this study, we found contradicting results. Some extracts are active on both gram positive and gram negative hke *Senna didymobotrya* DCM which was active against MRSA and *P. aeruginosa*, hence this can be considered as having broad spectrum of activity and present a potential agent for control of drug resistant strains. It is also good to note that, *Senna didmobotrya* is widely used among the Kipsigis for a wide range of conditions including; malaria, diarrhoea and skin infections hence, this practice is supported by the demonstrated broad spectrum of activity of its different extracts. Water extracts of *Senna didmobotrya* had good activity and support the traditional methods of water extraction of most of the medicinal plants.

Extracts from organic solvents had good activities as compared to water extracts, interestingly most medicinal plants are used as water extracts despite the low activities observed in this study (Gachathi, 1989; Kokwaro, 1976). This result agrees with the findings of Kigondu *et al.* (2009) which indicated that methanol extracts were more active as compared to water extracts. The activity could be explained by the ability of Methanol to extract both polar and non polar compounds hence more active compounds in the extracts.

The preliminary screening assays for antimicrobial activity can largely be considered as qualitative assays and are used for identifying the presence or absence of bioactive constituents in the extracts. However, these methods of assay offer little information on these compounds. The MIC is a quantitative assay and provides more information on the potency of the compounds present in the extracts. Thus, the MIC values of crude extracts of the two medicinal plants were determined so as to demonstrate the potency of the extracts against the selected strains of bacteria and fungi. The MIC of the crude extracts were has high as 100 mg mL^{-1} as compared to the standard drugs which is 0.5 mg mL^{-1} for both yeast and bacterial and 1.0 mg mL^{-1} for dermatophytes at 95% confidence interval ($p = 0.05$ level of significance). Although this was significantly lower than that of fluconazole ($p<0.01$) the extracts are promising since they are crude extract compared to pure compound of gentamycin and fluconazole. This is a clear indication that the active ingredient is present in low quantities which necessitate the use of large amounts of crude extracts to gain the desired therapeutic effects. The differences in bioactivities could be attributed to the facts that plants differ phytochemically and the extraction procedures could also affect or alter their composition. Absences of bioactivity does not warrant disapproval of ethnobotanical utilization of the plants, simply because it may suggest that the extracts are acting in an indirect way where active ingredients exist as a precursor requiring activation *in vivo*. Another reason is that since the

tradition herbal practitioners are more often than not using plants in combination to attain therapeutic effects, the extracts could be acting synergistically. Another reason is that, the herbalist is using the extracts in large quantities hence having therapeutic activities since the concentration of the active ingredients increases with the increasing quantity of the crude extracts. From this study, it is clear that activities vary greatly from plant to plant, solvent used for extraction and also on different. Among the Kipsigis community the extracts of *Senna didymobotrya* is widely used for skin conditions which may be caused by dermatophytes and bacteria. This study therefore provides scientific evidence of its efficacy against skin disease coursing microorganisms.

Thin layer chromatography: Phytochemical screening is important in determining the type of chemical constituents found in a given herbal drug or extract. The plants were screened using thin layer chromatography visualization reagents. According to the results of the phytochemical screening study, phenols were present in the two extracts. The *S. didymobotrya* methanol extract showed positive test for the presence of alkaloids and flavonoids. Terpenoids and Anthraquinones were present on *S. didymobotrya* dichloromethane extract and absent in the other plants extracts screened. The presences of flavonoids, phenolics or terpenoids in plants have been reported to be responsible for antimicrobial activity in plants (Nostro *et al.*, 2000). Flavonoids and flavonoid-derived plant natural products have long been known to function as antimicrobial defense compounds (Kazmi *et al.*, 1994). The different *in vitro* studies have also shown that they are effective antimicrobial substances against a wide spectrum of microorganisms (Schewe and Sies, 2003). For example, the quercetin and naringenin (Meng *et al.*, 2000) and the mixture of catechin compounds (found in oolong and green teas) (Rauha *et al.*, 2000) have been shown to exert antimicrobial activity against a wide range of microorganisms. Recent findings have also showed that ronane diterpenes, hugorosenone [3 β-hydroxyrosa-1, 15-dien-2-one and 18-hydroxy hugorosenone among others] from *H. casteneifolia* have both larvicidal and antifungal activity (Baraza *et al.*, 2008). The antifungal and antibacterial activity of the plants studied was attributed to the presences of these secondary metabolites. In plants, their role is to protect plants against microorganisms and insects, (Cowen, 2008).

***In vitro* toxicity (IC_{50}) of the two medicinal plant extracts:** Toxicity studies are very important in screening medicinal plants for safety. The extracts that had MIC of 50.0 mg mL^{-1} and below were considered potent and their cell toxicity were determined. The CC_{50} for the plants extracts was tested on 100 and 100 mg mL^{-1} concentrations to determine their safety. Cell toxicity of the extracts were categorized into; Toxic CC_{50} below 50% and safe CC_{50} above 50% (Zirihi *et al.*, 2005). Except *C. polycephala*, water extracts of the test plants generally had lower cytotoxicities than both methanol and DCM. The results are in agreement with other work done by Muthaura *et al.* (2007) on antimalaria properties of *Boscia angustifolia*. Cepleanu *et al.* (1994) also reported that the stem bark extracts from this plant had neither cytotoxicity nor brine shrimp lethality. These results seem to validate the traditional practice where herbs are extracted in boiled water (Gessler *et al.*, 1995). Most of the extracts tested had low toxicity (CC_{50} above 50%) against HELF cells, suggesting that they may be safe as antimicrobials. This result agrees with other studies done by Kigondu *et al.* (2009) on cell toxicity of medicinal plants.

Acute toxicity of *Senna didymobotrya* and *Cyathula polycephala* extracts: Acute toxicity was done on *Senna didymobotrya* DCM extracts and *Cyathula polycephala* water extracts since

they were found to be toxic to the cells. The extracts were considered safe when the death of mice was less than 50% which was observed at a lower dose of less than 5000 mg kg^{-1} (Zirihi *et al.*, 2005). It was found that *C. polycephala* extract is safe as an antimicrobial agent at both doses. These results are in agreement with the findings of Muthaura *et al.* (2007) who found that, the water extract was less toxic than the DCM extract. *Senna didymobotrya* is widely used among the Kipsigis community in treating and managing skin and diarrhoea infections. However, the method of preparation such as burning into ashes and then mixing with margarine for skin conditions and mixing with hot water and milk for stomach problems may be a way of reducing toxicity otherwise the population may be continuously exposed to cytotoxic plant.

CONCLUSION

*Senna Didymobot*rya was active against both gram positive and gram negative bacterial as well as fungal isolates; hence it has a broad spectrum of activity. Some extracts in this study has high activity against fungal strains as compared to bacteria but generally the bioactivities of all the extracts tested were generally good on bacterial isolates as compared to fungi. In both the cell and acute toxicity, it was observed that extracts at high concentration and at a high dose tend to be toxic as compared to lower concentration and lower doses. In particular, the *Senna didymobotrya* DCM extracts was toxic with LD_{50} between 1000 and 5000 mg kg^{-1}, therefore, for safety measures, herbs should be taken at lower concentration and lower doses. *Senna didymobotrya* is generally applied topically and mixed with milk or margarine for oral use; this is basically to reduce toxicity.

RECOMMENDATIONS

It is worth recommending that since some plants are potent both antibacterial and antifungal, further work should be done especially analysis and isolation of the compounds present as well as determination of their bioactivity. Bioassay of combinations of plant extracts that exhibited moderate and low activity should be carried out to establish any synergism between them. Since, this study was based on the plants part used by herbalist, it is worth recommending that other parts be studied. The plants that were found to be toxic to the mice should not be used as antimicrobial agents unless the herbal practitioners have other means of reducing their toxicity, alternatively they can be used at very low concentration and doses. Since some plants have good activities they should be formulated into different consumable forms as well as being incorporated into food as supplements. The plants should be conserved both *in situ* and *ex-situ*.

ACKNOWLEDGMENT

The authors acknowledge the research grants from International Foundation of Science which enabled this work to be done. The Director Kenya Medical Research Institute is also acknowledged.

REFERENCES

Baker, J.T., R.P. Boris, B. Carte, G.A. Cordell and D.D. Soejarto *et al.*, 1995. Natural product drug discovery and development: New perspectives on international collaboration. J. Nat. Prod., 5: 1325-1357.

Baraza, L.D., C.C. Joseph, J.J. Munissi, M.H. Nkunya, N. Arnold, A. Porzel and L. Wessjohann, 2008. Antifungal rosane diterpenes and other constituents of *Hugonia casteneifolia*. Phytochemistry, 69: 2000-2005.

Bii, C., K.R. Korir, J. Rugutt and C. Mutai, 2010. The potential use of *Prunus africana* for the control, treatment and management of common fungal and bacterial infections. J. Med. Plants Res., 4: 995-998.

Brun-Buisson, C., P. Legrang and A. Philipon, 1987. Transferase enzymic resistance to third generation cephalosporin using nosocomial outbreaks of multiresistance *Klebsiella pneumonia*. Lancet, 2: 302-306.

Cepleanu, F., M.O. Hamburger, B. Sordat, J.D. Msonthi, M.P. Gupta, M. Saadou and K. Hostettmann, 1994. Screening of tropical medicinal plants for molluscidal, larvicidal, fungicidal and cytotoxic activities and brine shrimp toxicity. Int. J. Pharmacogn., 32: 294-307.

Chhabra, S.C., F.C. Uiso and E.N. Mshiu, 1984. Phytochemical screening of Tanzanian medicinal plants. Part 1. J. Ethnopharmacol., 11: 157-179.

Cowen, L.E., 2008. The evolution of fungal drug resistance: Modulating the trajectory from genotype to phenotype. Nat. Rev. Microbiol., 6: 187-198.

Desta, B., 1993. Ethiopia traditional herbal drugs part II: Antimicrobial activity of 63 medicinal plants. J. Ethnopharmacol., 42: 129-139.

Elgayyar, M., F.A. Draughon, D.A. Golden and J.R. Mount, 2001. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. J. Food Prot., 64: 1019-1024.

Evans, W.C., 1997. Trease and Evans' Pharmacognosy. 14th Edn., W.B. Saunders, London, UK..

Ferraro, M.J., 2003. Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard. 8th Edn., National Committee for Clinical Laboratory Standards (NCCLS), Wayne, PA., USA., ISBN-13: 9781562384852, Pages: 34.

Gachathi, F.N., 1989. Kikuyu Botanical Dictionary of Plants Names and Uses. AMREF Printing Department, Nairobi, Kenya, Pages: 242.

Gessler, M.C., M. Tanner, J. Chollet, M.H.H. Nkunya and M. Heinrich, 1995. Tanzanian medicinal plants used traditionally for the treatment of malaria: *In vivo* antimalarial and *in vitro* cytotoxic activities. Phytother. Res., 9: 504-508.

Harborne, J.B., 1998. Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. 3rd Edn., Springer Publishers, New York.

Hooper, D.C., 2001. Emerging mechanisms of fluoroquinolone resistance. Emerg. Infect. Dis., 7: 337-341.

Kareru, J.O., 2007. Medicinal plants of Kenya. East Africa Literature Bureau Nairobi, Kenya.

Kazmi, M.H., A. Malik, S. Hameed, N. Akhtar and S.N. Ali, 1994. An anthraquinone derivative from *Cassia italica*. Phytochemistry, 36: 761-763.

Kigondu, E.V.M., 2007. Phytochemical and anti-parasitic activity studies of some selected Kenya medicinal plants. M.Sc. Thesis, Jomo Kenyatta University of Agriculture and Technology, Kenya.

Kigondu, E.V.M., G.M. Rukunga, J.M. Keriko, W.K. Tonui and J.W. Gathirwa *et al*., 2009. Anti-parasitic activity and cytotoxicity of selected medicinal plants from Kenya. J. Ethnopharmacol., 123: 504-509.

Kokwaro, J.O., 1976. Medicinal Plants of East Africa. East Africa Literature Bureau, Nairobi, Kenya.

Kurokawa, M., T. Hozumi, M. Tsurita, S. Kadota, T. Namba and K. Shiraki, 2001. Biological characterization of eugeniin as an anti-herpes simplex virus type 1 compound *in vitro* and *in vivo*. J. Pharmacol. Exp. Ther., 297: 372-379.

Meng, J.C., Q.X. Zhu and R.X. Tan, 2000. New antimicrobial mono and sesquiterpenes from *Soroseris hookeriana* subsp. *erysimoides*. Planta Med., 66: 541-544.

Michael, J.P., E.C. Chan, R.K. Noel and F.P. Merna, 2003. Microbiology. 5th Edn., Tata McGraw-Hill, New Delhi, India, pp: 627-748.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Method, 65: 55-63.

Mukherjee, P.K., 2002. Alternative Systems of Medicine. In: Quality Control Herbal Drugs: An Approach to Evaluation of Botanicals, Business Horizons, Mukherjee, P.K. (Ed.). New Delhi, India.

Muthaura, C.N., G.M. Rukunga, S.C. Chhabra, S.A. Omar and A.N. Guantai *et al*., 2007. Antimalarial activity of some plants traditionally used in Meru district of Kenya. Phytother. Res., 21: 860-867.

Njoroge, G.N. and R.W. Bussmann, 2007. Ethnotherapeautic management of skin diseases among the Kikuyus of Central Kenya. J. Ethnopharmacol., 111: 303-307.

Nostro, A., M.P. Germano, V. D'Angelo, A. Marino and M.A. Cannatelli, 2000. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett. Applied Microbial., 30: 379-384.

Rahman, S., R.B. Blok, H.H. Dahl, D.M. Danks and D.M. Kirby *et al*., 1996. Leigh syndrome: Clinical features and biochemical and DNA abnormalities. Ann. Neurol., 39: 343-351.

Rajakaruna, N., S.C. Harris and G.H.N. Towers, 2002. Antimicrobial activity of plants collected from Serpentine outcrops in Sri Lanka. Pharm. Biol., 40: 235-244.

Rauha, J.P., S. Remes, M. Heinonen, A. Hopia and M. Kahkonen *et al*., 2000. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int. J. Food Microbiol., 56: 3-12.

Schewe, T. and H. Sies, 2003. Flavonoids as protectants against prooxidant enzymes. Biol. Med., 34: 243-253.

Stein, A.C., M. Sortino, C. Avancini, S. Zacchino and G. von Poser, 2005. Ethnoveterinary medicine in the search of antimicrobial agents: Antifungal activity of *Pterocaulon* (Asteraceae). J. Ethanopharmacol., 99: 211-214.

Thompson, E.D., 1985. Drug Bioscreening: Fundamentals of Drug Evaluation Techniques in Pharmacology. Vol. 1, Graceway Publishing Co., Fresh Meadows, Queens, NY., USA., ISBN-13: 9780932126078, Pages: 164.

Twaij, H.A.A., A. Kery, N.K. Al-Khazraji, 1983. Some pharmacological, toxicological and phytochemical investigations on *Centaurea phyllocephala*. J. Ethnopharm., 9: 299-314.

WHO, 2002. WHO Monographs on Selected Medicinal Plants. Vol. 2, World Health Organization, Geneva, Switzerland, Pages: 246.

WHO, 2004. The world health report: The problem of antibiotic resistance. World Health Organization, Geneva, Switzerland.

Wanyoike, G.N., S.C. Chhabra, C.C. Lang, at-Thoruwa and S.A. Omar, 2004. Brine shrimp toxicity and antiplasmodial activity of five Kenyan medicinal plants. J. Ethnopharmacol., 90: 129-133.

Wilkins, K.M. and R.G. Board, 1989. Natural Antimicrobial Systems. In: Mechanisms of Action of Food Preservation Procedures, Gould, G.W. (Ed.). Elsevier Applied Science, London, UK., ISBN-13: 9781851662937, pp: 285-362.

Zirihi, G.N., L. Mambu, F. Guede-Guina, B. Bodo and P. Grellier, 2005. *In vitro* antiplasmodial activity and cytotoxicity of 33 West African plants used for treatment of malaria. J. Ethnopharmacol., 98: 281-285.