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Phytochemical analysis and antimicrobial activity of *Phytolacca dodecandra*, Cucumis aculeatus and Erythrina excelsa

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ABSTRACT

Three medicinal plants, Erythrina excelsa, Phytolacca dodecandra and Cucumis aculeatus, collected from Siaya and Migori District in Kenya, were screened for the presence of phytochemicals. These plants have diverse compounds including phenolics, terpenoids, anthraquinones, alkaloids and flavonoids, whereby the terpenoids were the most abundant. The antimicrobial activity of the hexane, dichloromethane, ethyl acetate, methanol and water extracts from the stem barks, root barks and the leaves of selected medicinal plants were assayed against eight microorganisms. In comparison with the standard antibiotics (chloramphenicol), the dichloromethane extract presented a high activity against Pseudomonas aeruginosa ATCC 27853 and a moderate activity on Salmonella typhi (Clinical isolate). The methanol extract presented significant activity against clinical fungal isolates, Microsporum gypseum and Trychophyton mentagrophytes. The hexane and ethyl acetate extract of Cucumis aculeatus leaves were active against P. aeruginosa ATCC 27853, whereas the dichloromethane extract of Erythrina excelsa had antibacterial activity against Staphylococcus aureus ATCC 25923. Hexane, dichloromethane and water extracts of Erythrina excelsa also showed activity against Pseudomonas aeruginosa ATCC 27853. These investigations shows that various extracts obtained from Erythrina excelsa, Phytolacca dodecandra and Cucumis aculeatus could be used in vitro to inhibit the growth of some important bacteria and fungi. The above results justify the reason why these medicinal plants have been and are being used to treat the fungal and bacterial infections by the Luo community. © 2012 International Formulae Group. All rights reserved.

Keywords: Phytochemicals, *Erythrina excelsa*, *Phytolacca dodecandra*, *Cucumis aculeatus*, antimicrobial properties.

INTRODUCTION

Plants have proved to be significant natural resources for medicines; documentation of their use in medicine originates from ancient times. Ethnobotanical plants provide a rich resource for natural drug

research and development (Nkunya, 2002; Kong et al., 2008). Medicinal plant-based drugs have the added advantage of being simple, effective and offering a broad spectrum of activity with greater emphasis on preventive action (Chin et al., 2006).

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Natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world. Well-known examples of plant-derived medicines include quinine, morphine, codeine, aspirin, atropine, reserpine and cocaine. Important anti-cancer drugs such as taxol from Taxus baccata and vincristine from Catharanthus roseus have been developed (En-Erick et al., 2002). Therefore, with emerging and re-emerging infections and spread of deadly, drug-resistant strains of organisms, plant-based drugs could provide both concepts of therapy as well as therapeutic agents to complement modern medicine especially in antibiotics resistant microbial pathogens. Thus, the search for antimicrobial agents is of the utmost importance.

In Kenya, the rural communities depend on plant resources mainly for herbal medicines (Kokwaro, 1993). Traditional healers claim that their medicine is cheaper and more effective than modern medicine. In developing countries, low-income people such as farmers, people of small isolated villages and native communities use folk medicine for the treatment of common infections. The plants *Phytolacca dodecandra*, *Cucumis aculeatus* and *Erythrina excelsa* are used in folk medicine in the treatment of several diseases.

Phytolacca dodecandra leaves are used for treating ringworms, roots and stem used for dysentery and other stomach disorders and the seeds are used as molluscidal. Some studies done on this plant have shown significant molluscicidal properties, but in concentrations far too high for use in practical applications (Allen-Gil and Aldea, 2003). Cucumis aculeatus fruits are used for treating abscesses in children, leaves and stem are used for treating dysentery. The cucubitacins metabolites which are biologically active and highly cytotoxic have been isolated from this plant (Neuwinger, 1994). Erythrina excelsa bark is used for gastrointestinal disorders,

amoebeasis and an anti-dote for snake-bites. However, up to date, little research has been done to investigate these traditionally used plants. Although numerous studies have been carried out on using natural products for screening antimicrobial activity (Matu and Van Staden 2003; Moshi et al., 2006), no attention has been given to *Phytolacca dodecandra*, *Cucumis aculeatus* and *Erythrina excelsa* antimicrobial activity. The main objective of these investigations was to establish the phytochemical profiles and relate the bioactivities to the ethnomedical use of these plants.

We report on our findings of some antibacterial and antifungal effects of various organic and aqueous extracts from leaves of *Phytolacca dodecandra*, *Cucumis aculeatus* and *Erythrina excelsa* against a wide range of pathogenic bacteria and dermatophyte strains.

MATERIALS AND METHODS Plant material

The plant materials for *Phytolacca* dodecandra, *Cucumis aculeatus* and *Erythrina* excelsa were collected from Siaya District and Migori District in Nyanza Province in December 2007. These were taken to Biology Department at the Catholic University of Eastern Africa (CUEA) for identification. The specimens of these plants have been kept in CUEA herbarium.

Extraction

The dried and powdered plant materials (100 g) were extracted using organic solvents and water. To obtain the organic extract, successive extraction was done using hexane, dichloromethane, ethyl acetate and methanol. The material was soaked at room temperature for 24 hours and then the extract filtered. The resultant extract was concentrated under reduced pressure at 40 °C using rotary evaporator. The water extract was then obtained by taking 50 g of plant material of each sample, weighed and soaked in distilled

water. This was then put into a water bath at a temperature of 60 °C for about one and a half hours, then filtered using cotton wool and stored in a deep-freezer to avoid moulds growing in it. The extract was freeze dried for three days. All the dried extracts both organic and aqueous were weighed so as to calculate the percentage yield of the various samples. The dried materials were stored at -20 °C (McCloud et al., 1988).

Phytochemical screening

The hexane, dichloromethane, ethyl acetate, methanol and aqueous extracts of Phytolacca dodecandra, Cucumis aculeatus and Erythrina excelsa were analyzed by qualitative method (thin layer chromatography on silica gel/UV detection at 365 nm) for presence of alkaloids. saponins, anthraquinones, flavonoids, phenol, and terpenoids (Orech et al., 2005; Edeoga et al., For the extracts of hexane, dichloromethane and ethyl acetate, the solvent system used to run the TLC was a mixture of dichloromethane and hexane at a ratio of 9:1 respectively. A different solvent system was used for the methanol extracts which was a mixture of dichloromethane and methanol in the ratio of 3:1 respectively. The plates were then visualized under Ultraviolet light at 364 to 235 nm to determine those extracts with fluorescing compounds. The plates were then sprayed or exposed to different reagents to identify the compounds present.

Bioassays

Screening for antibacterial activity

Antimicrobial activity was done at the Mycology Laboratories, Center for Microbiology Research - Kenya Medical Research Institute (KEMRI) following standard methods described in the Clinical Laboratory Standard Institute (CLSI). The procedure was done using disc diffusion method (Sing et al., 2002). The crude plant extracts were dissolved in DMSO and then

vortexes to enhance their solubility in the DMSO. The prepared plates were then labeled and then discs (Whatman 6 mm), 10 µl of the extract impregnated in every disc. Aseptically the disc was transferred to plates inoculated with 0.5 Mac Farland standard of the test bacteria. The plates were then incubated overnight at a temperature of 35 °C. The diameter of inhibition zones for all the extracts were measured and recorded. The activities of the extracts were tested against Methicilline Resistant Staphylococcus aureus ATCC 25923 (MRSA), Salmonella typhi (Clinical isolate), Pseudomonas aeruginosa ATCC 27853 and Escherichia coli ATCC 25922.

Screening for antifungal activity

This was done at Mycology laboratory, CMR, KEMRI, following standard methods. The procedures were done as described previously by disc diffusion method (Sing et al., 2002). The activity of the extracts were tested against Cryptococcus neoformans ATCC 66037, Candida albicans ATCC 90028, Microsporum gypseum KMCC Mg Trychophyton mentagrophytes KMCC TM 200. The crude plant extracts were dissolved in DMSO and then vortexed to enhance their solubility in the DMSO. The prepared plates were inoculated with 0.5 Mac Farland standard of the test fungi and disc impregnated with 10 µl of the test extracts were aseptically placed on the inoculated plates. The plates were incubated for 72 hours at 30 °C for filamentous fungi and 35 °C for 24 hours for yeasts. After incubation, the diameter of inhibition zones for the all the extracts were measured and recorded as an indicator for activity.

Serial dilution for the active extracts

The extracts which had activity against some of the bacteria and fungi were then subjected to serial dilution to determine the Minimum Inhibitory Concentration (MIC) for the extracts. Microdilution using microtitre plates were used for serial dilutions; for every bioactive extract, each well in the microtitre plates was filled with 10 μ l of DMSO. To the first whole 10 μ l of the extract was added and thoroughly mixed with the 10 μ l DMSO after which 10 μ l of the mixture was added to the second whole. This process was repeated to all the holes until to the eleventh hole while the twelfth hole acted as the control with only DMSO in it.

The dilutions from the wells were then subjected to bioactivity test using disc-diffusion method to determine the zones of inhibition. The activity of the extracts were compared to that of the standard drug; chloramphenicol for bacteria and fluconazole for fungi.

RESULTS

Phytochemical analysis

Most of the extracts of *Phytolacca* dodecandra had terpenoids except for the methanol leaf and stem bark extract. Most of the extracts had phenolics except the stem bark hexane extract. Most of the extracts did not indicate the presence of flavonoids, anthraquinones and alkaloids except for the hexane leaf extract and dichloromethane root bark extract indicated the presence of alkaloids.

The root barks of Phytolacca dodecandra showed highest concentration of terpenoids and phenolics as compared with the leaves and stems. In the leaves, terpenoids and phenolics had equal concentrations; the hexane extract had the highest concentration of terpenoid. Methanol extract indicated high concentration of phenolics and only hexane extract had alkaloids present. From the stem extract, phenolics were abundant among the compounds tested for. The ethyl acetate and hexane extracts had high concentrations of terpenoids as compared to the other extracts; dichloromethane extract had high concentration of phenolics as compared to the

other extracts. In the roots, there were a lot of terpenoids as compared to the other compounds tested for; hexane and Ethyl acetate extracts having the highest concentration of terpenoids and hexane extract had the highest concentration of phenolics as compared to the other extracts (Table 1).

Most of the leaf and stem extracts of *Cucumis aculeatus* indicated the presence of terpenoids and phenolics except for methanol leaf extract which did not indicate the presence of phenolics. However, most of the extracts did not indicate the presence of flavonoids, anthraquinones and alkaloids except for dichloromethane stem extract which indicated the presence of both anthraquinones and alkaloids; dichloromethane leaf extract indicated the presence of anthraquinones (Table 2).

Anthraquinones were not present in any of the extracts of *Erythrina excelsa*. Terpenoids and phenolics were present in most of the extracts except for ethyl acetate which did not indicate the presence of phenolics. Flavonoids were only detected in dichloromethane extract of this plant and alkaloids were found present in dichloromethane and methanol extracts only (Table 3).

Antibacterial and antifungal activities of test extracts of *Phytolacca dodecandra*

The hexane extracts did not have any activity against any of the bacterial isolates used in the study. The dichloromethane extract had high activity against *Pseudomonas aeruginosa*. ATCC 27853, moderate activity on clinical isolate of *Salmonella typhi* but no activity against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. Ethyl acetate extract had activity against *P. aeruginosa* ATCC 27853 but this was less than the activity of dichloromethane. The extracts had mild activity against clinical isolate of *Salmonella typhi* but no activity

against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. The methanolic extract had no activity while aqueous extract had activity against *P. aeruginosa* ATCC 27853 only (Table 4).

Dichloromethane, hexane, methanol and ethyl acetate extracts of leaves of *Phytolacca dodecandra* had no activity against the fungal isolates used in the study. Aqueous extract had moderate activity against *Microsporum gypseum* clinical isolate KMCC Mg 201 and *Trychophyton mentagrophytes* Clinical isolate KMCC TM 200 and mild activity against *Candida albicans* ATCC 90028.

Dichloromethane, hexane, methanol and ethvl acetate extracts of Phytolacca dodecandra leaves had no activity against the fungal isolates used in the study. Aqueous extract had moderate activity against Microsporum gypseum Clinical isolate KMCC Mg 201 and Trychophyton mentagrophytes Clinical isolate KMCC TM 200 and mild activities against Candida albicans ATCC 90028. The Phytolacca dodecandra leaf extracts had higher antibacterial activity as compared to antifungal activity (Tables 4 and 5). The methanol extract of Phytolacca dodecandra stem bark was the most active against Salmonella typhi and P. aeruginosa ATCC 27853. P. aeruginosa ATCC 27853 was the most susceptible bacterial isolate to dichloromethane, ethyl acetate, methanol and aqueous extracts (Table 5).

For the fungal isolates it, was only the methanol extract of *Phytolacca dodecandra* stem bark which had antifungal activities except for *Candida albicans* ATCC 90028 in which there was no activity at all as indicated in Table 5.

Dichloromethane and ethyl acetate extracts of the roots of *Phytolacca dodecandra* were active against *P. aeruginosa* ATCC 27853. The aqueous extract had very mild activity against *Escherichia coli* ATCC 25922 while ethyl acetate also had mild

activity against *Salmonella typhi*. Hexane and methanol extracts had no antibacterial activity against any of the bacterial isolates used in the study.

Ethyl acetate of Phytolacca dodecandra roots had very mild antifungal activity against all the four fungal extracts used in the study, the methanol extract had antifungal activity all the four fungal isolates used in the study and this extract had higher activity Microsporum gypseum Clinical isolate KMCC Mg 201 and Trychophyton mentagrophytes Clinical isolate KMCC TM 200 (Table 6). The aqueous extract had mild activity on Cryptococcus neoformans ATCC 66037 and a moderate antifungal activity on Candida albicans ATCC 90028 as shown in Table 6. The extracts from the roots of Phytolacca dodecandra had greater antifungal activity as compared to the antibacterial activity.

Antibacterial and antifungal activity of test extracts of *Cucumis aculeatus*

The hexane and methanol extracts of Cucumis aculeatus leaves had no antifungal activity; the aqueous extract exhibiting antifungal activity only on Trychophyton mentagrophytes Clinical isolate KMCC TM 200 (Table 7). Dichloromethane extract had mild antifungal activity against Microsporum gypseum Clinical isolate KMCC Mg 201 and Trychophyton mentagrophytes Clinical isolate KMCC TM 200; ethyl acetate had mild activity on Microsporum gypseum Clinical isolate KMCC Mg 201 and Trychophyton mentagrophytes Clinical isolate KMCC TM 200. The extracts from the leaves of Cucumis aculeatus had greater antibacterial activity as compared to the antifungal activity.

From the stem extracts of *Cucumis* aculeatus, ethyl acetate extract had greater antibacterial activity against *Pseudomonas* aeruginosa ATCC 27853, very mild activity against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 and mild

activity on Salmonella typhi Clinical isolate. had Dichloromethane activity Pseudomonas aeruginosa ATCC 27853; hexane had activity against Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923 and Pseudomonas aeruginosa ATCC 27853. Methanol had activity against Pseudomonas aeruginosa ATCC 27853 and the aqueous extract had activity against Salmonella typhi Clinical isolate (Table 8). The hexane and methanol stem extracts of Cucumis aculeatus had no antifungal activity against the fungal isolates used in this study. However, dichloromethane, methanol and aqueous extracts had antifungal against Microsporum gypseum Clinical isolate KMCC Mg 201 and Trychophyton mentagrophytes Clinical isolate KMCC TM 200 (Table 9).

Antibacterial and antifungal activity of test extracts of *Erythrina excelsa* stem bark

Dichloromethane, ethyl acetate and methanol extract had antibacterial activity against *Staphylococcus aureus* ATCC 25923; hexane, dichloromethane and aqueous had activity against *Pseudomonas aeruginosa* ATCC 27853 (Table 10). *Erythrina excelsa* stem bark-dichloromethane extract.

Minimum Inhibitory Concentration (MIC)

All the extracts in this investigation which showed bioactivity against any of the microorganisms used in the study were further subjected to serial dilution procedure. These extracts included Phytolacca dodecandra stem-methanol extract, leavesdichloromethane extract. roots-methanol extract, stem-dichloromethane extract, stemethyl acetate extract, root-ethyl acetate extract, roots-dichloromethane extract. Cucumis aculeatus leaves-ethyl acetate extract, stemethyl acetate extract, Phytolacca dodecandra leaves-water extract, Erythrina excelsa stem bark-dichlormethane extract and stem barkdichloromethane extract.

Some of the extracts did not show any bioactivity against any of the bacteria and fungi tested thus their MIC could not be These included Phytolacca determined. dodecandra leaves ethyl acetate extract which showed no bioactivity against Salmonella typhi and Escherichia coli ATCC 25922. Phytolacca dodecandra leaves and roots water extracts showed no bioactivity against Candida albicans ATCC 90028. Phytolacca doecandra leaves dichloromethane and methanol extracts showed no activity against Salmonella typhi Clinical isolate. Phytolacca dodecandra roots ethyl acetate extract showed no activity against Cryptococcus neoformans ATCC 66037. Cucumis aculeatus leaves and stem ethyl acetate extracts and Cucumis aculeatus stem water extract showed no bioactivity against Salmonella typhi Clinical isolate.

Phytolacca dodecandra stem methanol extract had higher antibacterial activity against P. aeruginosa ATCC 27853 with an MIC of 0.8625 mg/ml while for Cryptococcus neoformans ATCC 66037 the MIC was 5.5 mg/ml. Phytolacca dodecandra roots dichloromethane extract had an MIC of 0.3125 mg/ml with a zone of inhibition of 7 mm for Pseudomonas aeruginosa ATCC 27853. Cucumis aculeatus leaves ethyl acetate extract had an MIC of 0.9 mg/ml with a zone of inhibition of 8 mm for Pseudomonas aeruginosa ATCC 27853 but no activity for Salmonella typhi Clinical isolate. Cucumis aculeatus stem ethyl acetate extract had MIC of 1.45 mg/ml with a zone of inhibition of 7 mm for Pseudomonas aeruginosa ATCC 27853. Phytolacca dodecandra leaves water extract had MIC of 3.4 mg/ml with a zone of inhibition of 8 mm. Cucumis aculeatus stem hexane extract had MIC of 14.4 mg/ml with a zone of inhibition of 7 mm. Erythrina excelsa stem bark dichloromethane extract had MIC of 0.163 mg/ml and a zone of inhibition of 7 mm.

Table 1: Results of phytochemical screening of *Phytolacca dodecandra*.

Plant parts	Compounds	Hexane	Dichloromethane	Ethyl acetate	Methanol
Leaves	Terpenoids	++++	+	+++	-
	Phenolics	+	++	++	+++
	Flavonoids	-	-	-	-
	Anthraquinones	-	-	-	-
	Alkaloids	++	-	-	-
Stem bark	Terpenoids	+	+	+++	-
	Phenolics	-	+++	+	++
	Flavonoids	-	-	-	-
	Anthraquinones	-	-	-	-
	Alkaloids	-	-	-	-
Root bark	Terpenoids	+++	++	+++	++
	Phenolics	++++	+	++	++
	Flavonoids	-	-	-	-
	Anthraquinones	-	-	-	-
	Alkaloids	- 1'	-	-	-

⁻ not determined; + low concentration; ++ medium concentration; +++ high concentration

 Table 2: Phytochemical screening results for Cucumis aculeatus.

Plant parts	Compounds	Hexane	Dichloro- methane	Ethyl acetate	Methanol
Leaves	Terpenoids	+++	++	++	++
	Phenolics	++++	++	++	-
	Flavonoids	-	-	-	-
	Anthraquinones	-	+	-	-
	Alkaloids	-	-	-	-
Stem	Terpenoids	+++	+	+++	++
	Phenolics	+++	++	+	++
	Flavonoids	-	-	-	-
	Anthraquinones	-	++	-	-
	Alkaloids	-	++	-	=

⁻ not determined; + low concentration; ++ medium concentration; +++ high concentration; ++++ very high concentration.

⁺⁺⁺⁺ very high concentration.

Table 3: Phytochemical screening results of *Erythrina excelsa* (Root bark).

Compounds	Hexane	Dichloromethane	Ethyl acetate	Methanol
Terpenoids	++++	+	+	+
Phenolics	+++	+	-	+++
Flavonoids	-	++	-	-
Anthraquinones	-	-	-	-
Alkaloids	_	++	-	++

⁻ not determined; + low concentration; ++ medium concentration; +++ high concentration; ++++ very high concentration.

Table 4: Antibacterial activity of test extracts of *Phytolacca dodecandra*.

Test strains/Plant part	Solvents/ Zones of inhibition and MIC (mg/ml)						
	Hexane	Dichloromethane	Ethyl acetate	Methanol	Water		
Leave extract							
Salmonella typhi (Clinical isolate)	-	10(N/A)	8(N/A)	-	-		
Escherichia coli ATCC 25922	-	6.5(N/A)	7(N/A)	-	6.5(N/A)		
Staphylococcus aureus ATCC 25923	-	-	-	6.5(N/A)	-		
Pseudomonas aeruginosa ATCC 27853	-	30(12)	14(N/A)	21(1.4)	17(1.4)		
Stem bark							
Salmonella typhi Clinical isolate	-	-	8	13	-		
Escherichia coli ATCC 25922	-	-	-	-	-		
Staphylococcus aureus ATCC 25923	-	-	-	-	-		
Pseudomonas aeruginosa ATCC 27853	-	22 (3.8)	22 (3.8)	50 (14)	11 (N/A)		
Root							
Salmonella typhi (Clinical isolate)	-	-	7 (N/A)	-	-		
Escherichia coli ATCC 25922	-	-	-	-	6.5 (N/A)		
Staphylococcus aureus ATCC 25923	-	-	-	-	-		
Pseudomonas aeruginosa ATCC 27853	-	20 (2.5)	22 (3.5)	-	-		

^{-:} No activity; (N/A): MIC not determined due to low activity.

Table 5: Antifungal activity of extracts of *Phytolacca dodecandra*.

	Solvents/Zones of inhibition and MIC (mg/ml)						
Test strains / Plant part	Hexane	Dichloromethane	Ethyl acetate	Methanol	Water		
Leaves							
Candida albicans ATCC 90028	-	-	-	-	8(N/A)		
Microsporum gypseum (Clinical isolate KMCC Mg 201)	-	-	6.5(N/A)	7(N/A)	14(110)		
Cryptococcus neoformans ATCC 66037	-	-	-	-	-		
Trychophyton mentagrophytes (Clinical isolate KMCC TM 200)	-	-	6.5(N/A)	7(N/A)	14(3.4)		
Stem bark							
Candida albicans ATCC 90028	-	-	-	-	-		
Microsporum gypseum Clinical isolate KMCC Mg 201	-	-	-	9(N/A)	-		
Cryptococcus neoformans ATCC 66037	-	-	-	8(14)	-		
Trychophyton mentagrophytes Clinical isolate KMCC TM 200	-	-	-	9(N/A)	-		
Root							
Candida albicans ATCC 90028	-	-	8	9	10 (N/A)		
Microsporum gypseum Clinical isolate KMCC Mg 201	-	-	7 (N/A)	16	-		
Cryptococcus neoformans ATCC 66037	-	-	7 (N/A)	10 (3.4)	8 (N/A)		
Trychophyton mentagrophytes Clinical isolate KMCC TM 200	-	-	7 (N/A)	16 (3.4)	-		

^{-:} No activity; (N/A): MIC not determined due to low activity.

Table 6: Antibacterial activity of test extracts of *Cucumis aculeatus* leaves.

Test strains	Zones of inhibition and MIC (mg/ml)						
	Hexane	Dichloro- methane	Ethyl acetate	Methanol	Water		
Salmonella typhi Clinical isolate	-	-	8 (N/A)	-	-		
Escherichia coli ATCC 25922	-	-	6.5 (N/A)	-	-		
Staphylococcus aureus ATCC 25923	-	8 (N/A)	-	8 (N/A)	-		
Pseudomonas aeruginosa ATCC 27853	16 (N/A)	-	38 (7.8)	9 (N/A)	12 (N/A)		

^{- :} No activity; (N/A): MIC not determined.

Table 7: Antifungal activity of test extracts of *Cucumis aculeatus* leaves.

	Zones of inhibition and MIC (mg/ml)					
Test strains	Hexane	Dichloro- methane	Ethyl acetate	Methanol	Water	
Candida albicans ATCC 90028	-	-	-	-	-	
Microsporum gypseum Clinical isolate KMCC Mg 201	-	7 (N/A)	7 (N/A)	-	-	
Cryptococcus neoformans ATCC 66037	-	-	-	-	-	
Trychophyton mentagrophytes Clinical isolate KMCC TM 200	-	7 (N/A)	10 (N/A)	-	8	

^{-:} No activity; (N/A): MIC not determined.

Table 8: Antibacterial activity of test extracts of *Cucumis aculeatus* stem.

	Zones of inhibition and MIC (mg/ml)						
Test strains	Hexane	Dichloro- methane	Ethyl acetate	Methanol	Water		
Salmonella typhi Clinical isolate	-	-	8 (N/A)	-	10 (N/A)		
Escherichia coli ATCC 25922	10.5 (N/A)	-	6.5 (N/A)	-	-		
Staphylococcus aureus ATCC 25923	10.5 (7.2)	-	6.5 (N/A)	-	-		
Pseudomonas aeruginosa ATCC 27853	14 (N/A)	18 (N/A)	50 (5.8)	7 (N/A)	-		

^{- :} No activity; (N/A): MIC not determined.

Table 9: Antifungal activity of test extracts of Cucumis aculeatus stem.

	Zones of inhibition and MIC (mg/ml)					
Test strains	Hexane	Dichloro- methane	Ethyl acetate	Methanol	Water	
Candida albicans ATCC 90028	-	-	-	-	-	
Microsporum gypseum Clinical isolate KMCC Mg201	-	7 (N/A)	10 (N/A)	-	8 (N/A)	
Cryptococcus neoformans ATCC 66037	-	-	-	-	-	
Trychophyton mentagrophytes Clinical isolate KMCC TM 200	-	7 (N/A)	10 (N/A)	-	8 (N/A)	

^{- :} No activity; (N/A): MIC not determined.

Table 10: Antibacterial activity of test extracts of *Erythrina excelsa* stem bark.

	Zones of inhibition and MIC (mg/ml)						
Test strains	Hexane	Dichloro- methane	Ethyl acetate	Methanol	Water		
Salmonella typhi Clinical isolate	-	-	-	-	-		
Escherichia coli ATCC 25922	-	-	-	-	-		
Staphylococcus aureus ATCC 25923	-	9 (0.16)	8 (N/A)	7 (N/A)	-		
Pseudomonas aeruginosa ATCC 27853	8 (N/A)	12 (N/A)	-	-	18 (N/A)		

^{-:} No activity; (N/A): MIC not determined.

DISCUSSION

From the extracts of root, stem barks and leaves of the *P. dodecadra, C. aculeatus and E. excelta*, phenolics and terpenoids were the most abundant among the compounds tested for. Flavonoids were identified mostly in *E. excelta* which is a confirmation of the presence of these comounds in *Erythrina* species (MI, 2010). Flavonoids are also widely used as antiplasmodial agents (Abiy, 2003). Thus, confirming the rationalization for these traditional use of these herbal remedies in treating microbial and other conditions

among the Nyanza community (Kokwaro, 1993). The antibacterial activity against *Staphylococcus aureus* ATCC 25923 could be likely due to the presence of the flavonoids.

From the results above, it was also clear that the plants *Phytolacca dodecandra*, *Cucumis aculeatus* and *Erythrina excelsa* could be the most reliable antimicrobial plants since their activity was consistent. Even after serial dilution, some extracts did not loose their activity. Their mild antibacterial and antifungal activity is a clear indication of their potential antimicrobial agents, especially if

the compounds could be isolated and tested. The Phytolacca dodecandra stem MIC of 4 µg/ml for P. aeruginosa ATCC 27853 with a minimum zone of inhibition of 22 mm and an MIC of 2 µg/ml for Escherichia coli ATCC 25922 with a minimum zone of inhibition of 26mm relates well with the tradition use of this plant. For example, the traditional use of Phytolacca dodecandra leaves for treatment ringworms, roots and stem used for dysentery and other stomach disorders and the seeds are used as molluscidal, could be an effect of the presence of phenolics and terpenoids (Merkl, 2010; Kisangau, 2007). A few of the alkaloids were detected and these could be an indicator of synergism that could be causing these bioactivities.

Conclusion

Plants investigated have diverse compounds including phenolics, terpenoids, anthraquinones, alkaloids and flavonoids. These compounds are not evenly distributed in the plants. Phytolacca dodecandra extracts demonstrated antibacterial activity against Salmonella typhi Clinical isolate and Pseudomonas aeruginosa ATCC 27853 and antifungal activity against Microsporum gypseum Clinical isolate KMCC Mg 201 and Trychophyton mentagrophytes Clinical isolate KMCC TM 200 but very mild antifungal activity against Candida albicans ATCC 90028 and Cryptococcus neoformans ATCC 66037. Cucumis aculeatus extracts demonstrated antibacterial activity against Salmonella typhi Clinical isolate, Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923 and Pseudomonas aeruginosa ATCC 27853. Cucumis aculeatus extracts also demonstrated antifungal activity against Microsporum gypseum Clinical isolate KMCC Mg201 and Trychophyton mentagrophytes Clinical isolate KMCC TM 200. Extracts from Erythrina excelsa demonstrated antibacterial activity against Staphylococcus aureus ATCC 25923 and P. aeruginosa ATCC 27853.

The above results justify the reason why these medicinal plants have been and are

being used to treat the fungal and bacterial infections by the Luo community. These three plants under investigation have shown bioactivity against some fungi and bacteria. Further studies of active metabolites will be highly appreciated. However, there is also need to encourage the community to domesticate these medicinal plants.

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REFERENCES

- Abiy Y, Derese S, Irungu B, Midiwo JO, Waters NC. 2003. Flavonoids and isoflavonoids with antiplasmodial activities from the root bark of Erythrina abyssinica. *Planta Medica*, **69**(7): 658-661.
- Allen-Gil S, Aldea M. 2003. Pokeweed (Phytolacca americana) as a Potential Molluscicide for Use in Aquaculture. Biology Department, Ithaca College: New York, USA.
- Chin Y, Balunas MJ, Chai HB, Kinghorn AD. 2006. Drug discovery from natural sources. *AAPS J.*, **8**: E239-E253.
- Edeoga HO, Okwu DE, Mbaebie BO. 2005. Phytochemical constituents of some Nigerian medicinal plants. *Afric. J. Biotech.*, **4**(7): 685–688.
- En-Erick W, Dudtshoorn B, Gericke N. 2002. *Medicinal Plants of South Africa*. Briza Publications: Pretoria, South Africa; 8.
- Kisangau DP, Hosea KM, Joseph CC, Lyaruu HVM. 2007. *In Vitro* Antimicrobial Assay of Plants Used in Traditional Medicine in Bukoba Rural District, Tanzania. *African Journal of Traditional*, *Complementary and Alternative Medicines*, **4**(4): 510–523.

- Kokwaro JO. 1993. *Medicinal plants of East Africa* (2nd edn). Kenya Literature Bureau: Nairobi, Kenya.
- Kong JM, Goh NK, Chia LS, Chia TF. 2003. Recent Advances in traditional plant drugs and orchids. *Acta Pharmacol. Sin.*, **24**: 7-21.
- Matu EN, Van Staden J. 2003. Antibacterial and anti-inflammatory activities of some plants used for medicinal purpose in Kenya. *J. Ethnopharmacology*, **87**(1): 35-41.
- Merkl R, Iveta Hrádková I, Vladimír FJŠ. 2010. Antimicrobial and Antioxidant Properties of Phenolic Acids Alkyl Esters. *Czech J. Food Sci.*, **28** (4): 275–279.
- MI A, Ahmed Z, Waffo AF, Ali MS. 2010. .Flavonoids from *Erythrina vogelii*

- (Fabaceae) of Cameroon. *Nat. Prod. Commun.*, **5**(6): 889-892.
- Neuwinger HD. 1994. Poisons and Drug-Chemistry, Pharmacology, Toxicology. African ethnobtany, Chapman and Hall: Germany.
- Nkunya MHH. 2002. Natural chemicals for diseases and insect management. Professoral Lecture, Department of Chemistry, University of Dar es Salaam: Tanzania.
- Orech FO, Akenga T, Ochora J, Friis H, Aagaard-Hansen J. 2005. Potential toxicity of some leafy vegetables consumed in Nyang'oma Division, Western Kenya. *Afri. J. Food Nutrit. Sci.*, 5(1): 1–13.