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Antimicrobial activities of *Clutia abyssinic* and *Erythrina abyssinica* plants extracts used among the Kipsigis community of Bomet district in Kenya

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ABSTRACT

The use of plants for medicinal purposes is an important part of the culture and tradition in Africa and the world at large. Up to 80% of the world population depends directly on the traditional medicine for the primarily health care. The objective of this study was to collect, extract and determine antimicrobial activities of *Clutia abyssinic* and *Erythrina abyssinica* plants. The two plant samples were collected from Bomet District. Taxonomical identification was done at East African Herbarium. Leaves and stem bark were air dried and separately grounded into fine powder. The powdered material was extracted with Hexane, dichloromethane, methanol and water. Extracts of the two plants were screened against bacterial and fungal isolates. The highest inhibition zone diameters recorded was 23.0 mm from hexane extract of *E. abyssinica* against *Trichophyton mentagrophyte*. The solvents used for extraction were used as negative control while fluconazoles and gentamycin were used as standards. The standards drugs inhibition zone diameter was between 16mm and 20mm which was comparable well with that of the plant extracts. The *E. abyssinica* hexane extract was the most active against *Microsporum gypseum* with minimum inhibitory concentration of 6.25mg/ml. The results were found to be significant at p value 0.005. The antifungal and antibacterial activity of the plants under investigation demonstrated support for the claimed antimicrobial uses of the plants in the traditional medicine and provides scientific prove for their medicinal values.

KEYWORDS

Medicinal plants;
Antifungal;
Antibacterial;
Antimicrobials;
Traditional medicine.

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[7]. Herbal drugs are prepared from various parts of the plant such as leaves, stem, roots, seeds, tubers or exudates[14]. The *C. abyssinic* plant belong to the family Euphorbiaceae. The plant extract

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is used traditionally in preparation of malaria remedy; leaves are used as an oral decoction for the treatment of hepatitis, externally as a local anti-inflammatory and as a medication for sores Vlietinck[18]. Alcohol extract of *Clutia richardiana* exhibited activity against both gram positive and gram negative bacteria[9,15]. The antimicrobial properties of this plant have not been determined in our local environment despite the fact that it has been widely studied in Rwanda and Tanzania as documented by Hugo[9] and Vlietinck[18], Boiled leaves of *C. abyssinica* are used traditionally to treat malaria while boiled leaves and roots are used for headaches and stomach problems[11]. The leaves and bark are burned into ashes, mixed with margarine and used traditionally to treat oral thrash by the Kipsigis community in Kenya. The plant *E. abyssinica* belong to the family Leguminosae-Papilionoideae. It is a flowering plant which is distributed in tropical and subtropical regions of the world. The seeds contain potent erythrina alkaloids, which are used for medicinal and other purposes by indigenous peoples. The main active compounds in this genus generally seem to be alkaloids, such as scoulerine, erysodin and erysovin (namely in *E. flabelliformis*), and the putative anxiolytic erythravine (isolated from Mulungu, *E. mulungu*). It is used traditionally by the Kipsigis people to treat skin conditions and trachoma. Antimicrobial properties of other species belonging to this genus have been studied e.g. *E. subumbrans,* by Thitima et al.[17], and Abiy et al.[1]. However, antimicrobial properties of *E. abyssinica* from Bomet District have not been determined.

MATERIALS AND METHODS

Collection of the plants from the field

Two Kenyan medicinal plants *Erythrina abyssinica* and *Clutia abyssinic* were collected from Bomet District forest. 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 both skin condition and stomach problem. The study plant were photographed and collected for authentification at the East African Herbarium. The samples for extractions were collected in paper bags and taken to the Medical chemistry Laboratory, Centre for Traditional Medicine and Drug Research for processing and extractions.

Extraction of plant materials

Plants materials were dried at room temperature, grounded into a fine powder using laboratory grinding mill 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 done successively using hexane, dichloromethane and methanol where the plant materials were soaked in 1000ml conical flasks for 12 hours. The extracts were filtered using Whatman No. 1 filter paper and solvents removed through evaporation under reduced pressure at 45^0C using a rotary evaporator. The extracts were kept in stoppered sample vials at 4^0 C until they were used[2,4,12]; Total water extract of each plant material was done by soaking a weighed amount of the dry powder (27-50g) in distilled water and shaking it for two hours with an electric shaker at 65^0C. 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.

Test strains

Gram positive bacterial

Staphylococcus aureus ATCC 25923 and Methicillin resistant *Staphylococcus aureus* (MRSA)

Gram negative bacterial

Escherichia coli 0157:H7 ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumonia.* (Clinical isolate)

Fungi isolates

Yeast: *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 90029, *Candida krusei* ATCC 90026 and *Cryptococcus neoformance* ATCC 9002

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 37^0C for 12 – 18 hours to obtain freshly growing strains. Yeast and molds were subcultured onto Sabaraud Dextrose Agar no. CM 004 (Oxoid Ltd, Basingstoke, Hampshire, England). Each media was prepared according to the manufacturers instructions. Yeasts were incubated for 24 hours while molds were incubated for 72 hours at 30^0C to obtain freshly growing culture.

Preparation of McFarland standard

Exactly 0.5 McFarland equivalent turbidity standards was prepared by adding 0.6ml of 1% barium chloride solution ($BaCl_2.2H_2O$) to 99.4ml of 1% sulphuric acid solution (H_2SO_4) and mixed thoroughly. A small volume of the turbid solution was transferred to capped 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 $1x10^{-6}$ colony forming units (CFU)/ml[2].

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 1ml of the appropriate solvents and 10 µl of the mixture was impregnated onto 6mm sterile filter paper disk and air dried. The disks were placed aseptically onto the inoculated plates. The bacterial and yeast cultures was incubated at 37^0C for 24 and 48 hours respectively. Dermatophytes cultures were incubated at 25^0C for 3-7 days after which the inhibition zone diameter was measured in millimeters and recorded against the corresponding concentrations as described by Elgayyar et al.[5]. Positive controls were set against standard antibiotics and antifungal drugs 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. This method is recommended by the National Committee for Clinical Laboratory Standards now Clinical Laboratory Standard Institute (CLSI). 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 from10^1, 10^2, 10^3……..$10^{.10}$ The final volume in each well was 100 µl. The wells were inoculated with 5µl of microbial suspension. The yeast and bacteria were incubated at 37^0C for 24 hours while molds were incubated at 25^0C 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[13]. Wells that was not inoculated was set to act as negative control for comparison. All the experiments were done in triplicates and average results were recorded.

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 at 0.05 level of confidence.

RESULTS AND DISCUSSION

The results obtained in this study indicate a considerable difference in antimicrobial activity of different extracts from the two selected plant species. The results of *C. abyssinica* indicates that the dichloromethane (DCM) extract was the most active against *P. aeruginosa* with inhibition zone diameters of 15.0 mm and the methanol extract had the least activity against *P. aeruginosa* with inhibition zone diameters of 8.0 mm. Methanol extracts for *C. abyssinica* recorded the lowest minimum inhibition concentration of <0.1953 mg/ml against *P. aeruginosa*,. The plants dichloromethane extracts recorded an MIC of 50.0 mg/ml against *P. aeruginosa.* Minimum inhibitory concentrations were not performed on fungal isolates since there was no activity recorded in the initial screening test.

Erithrina abyssinica hexane extract was active against *P. aeruginosa* with inhibition zone diameters of

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11.0 mm. It had the highest activity with inhibition zone diameter of 23.0 mm against *M. gypseum*. The dichloromethane extract was active against MRSA, *P. aeruginosa* and *K. pneumonia* with 10.0, 12.0 and 11.0 mm zone diameters respectively. The extract had different activities against the test fungal isolates as follows, 8.0, 7.5, 14.0 and 20.0 mm zone sizes against *C. parapsilosis, C.krusei, T. mentagrophyte* and *M.gypseum*, respectively. Methanol extract was active against MRSA, *P. aeruginosa* and *K. pneumoniae* with 9.0, 7.0 and 13.0 mm inhibition zones diameters. The water extracts had no activities against all the isolates tested. The plant recorded MIC ranging from 100.0 mg/ml to 6.25 mg/ml. The *E. abyssinica* methanol extract recorded the lowest MIC of 6.25 mg/ml against MRSA. Although this was significantly higher than that of gentamycin ($p < 0.001$) it is a promising plant extract given that it was a crude extract compared to pure compound of the standard drugs. The MIC for the fungi isolates ranged from 100 mg/ml to 6.25 mg/ml. The hexane extracts had the lowest MIC of 6.25 mg/ml against *M. gypseum*. 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[11]. Elsewhere studies have shown that *C.*

TABLE 1 : Antibacterial activity of different extracts of *Clutia abyssinica* and *Erythrina abyssinica* against selected strains of bacteria

Plants	Test samples (extracts/drug)	Conc mg/ml	Inhibition Zone diameters in millimeter for each *Test organisms*				
			S. aureus	MR*S. aureus*	*P. aeruginosa*	*E. coli*	*K. pneumoniae*
Clutia abyssinica	Hexane	100.0	6.0	6.0	6.0	6.0	6.0
	DCM	100.0	6.0	6.0	15.0	6.0	6.0
	Methanol	100.0	6.0	6.0	8.0	6.0	6.0
	Water	100.0	6.0	6.0	6.0	6.0	6.0
Erythrina abyssinica	Hexane	100.0	6.0	6.0	11.0	6.0	6.0
	DCM	100.0	6.0	10.0	12.0	6.0	11.0
	Methanol	100.0	10.0	9.0	7.0	6.0	13.0
	Water	100.0	6.0	6.0	6.0	6.0	6.0
- Control	DMSO	100.0	6.0	6.0	6.0	6.0	6.0
+ Control	Gentamycin	100.0	19.0	9.0	21.0	19.0	17.0
- Control	Water	100.0	6.0	6.0	6.0	6.0	6.0

TABLE 2 : Antifungal activities of different extracts of *Erythrina abyssinica* and *Clutia abyssinica* against selected strains of fungi

Plant	Test samples (extracts/drug)	Conc mg/ml	Inhibition Zone diameters in millimeter					
			CRPT	*CA*	*CP*	*CK*	TM	MG
Erythrina abyssinica	Hexane	100.0	6.0	6.0	6.0	6.0	6.0	23.0
	DCM	100.0	6.0	6.0	8.0	7.5	14.0	20.0
	Methanol	100.0	8.0	10.0	9.0	8.5	12.0	17.0
	Water	100.0	6.0	6.0	6.0	6.0	6.0	6.0
Clutia abyssinica	Hexane	100.0	6.0	6.0	6.0	6.0	6.0	6.0
	DCM	100.0	6.0	6.0	6.0	6.0	6.0	6.0
	Methanol	100.0	6.0	6.0	6.0	6.0	6.0	6.0
	Water	100.0	6.0	6.0	6.0	6.0	6.0	6.0
- control	DMSO	100.0	6.0	6.0	6.0	6.0	6.0	6.0
+ control	Fluconazole	30.0	23.0	27.0	24.0	26.0	20.0	20.0
- control	Water	100.0	6.0	6.0	6.0	6.0	6.0	6.0

***C RPT- Cryptococcus neoformans, CA- Candida albicans, CP-Candida parapsilosis, CK- Candida krusei, TM- Trichophyton mentagrophyte, MG-Microsporum gyepseum, DMSO- Dimethylsulfoxide.**

TABLE 3 : Minimum inhibitory concentration of *E. abyssinica* and *C. abyssinica* extracts against bacteria isolates

Plants	Test samples (extracts/drugs)	Test organism	*MIC (mg/ml)
Erithrina abyssinica	Hexane	*P. aeruginosa*	50.0
	DCM	*P. aeruginosa*	50.0
		MRSA	50.0
		K.pneumo	100.0
		S. aureus	25.0
	Methanol	*P. aeruginosa*	100.0
		MRSA	6.25
		K. pneumo	25.0
Clutia abyssinica	DCM	*P. aerugisa*	50.0
	Methanol	*P. aerugisa*	<0.1953
Standard	Gentamicin	All tested bacteria	0.5

***Std MIC in ug/ml**

abyssinica extracts have potent antimalaria activities[10], this phenomena could explain the low activitiy exhibited by this extracts against some bacterial and fungi. This result agrees with the findings of Elizabeth et al.[6] which indicated that methanol extracts were more active as compared to water extracts. Absence of in-vitro activity does not warranty disapproval of the ethanobotanical utilization has this suggest extracts acting in an indirect way where active ingredients exist as a precursor requiring activation in-vivo.

TABLE 4 : Minimum inhibitory concentration (MIC) of *E. abyssinica* extracts against fungal isolates

Plant	Test samples (extracts/drug)	Test organism	MIC (mg/ml)
Erithrina abyssinica	Hexane	*M. gypseum*	6.25
		T. mentagrophyte	25.0
	Dichloromethane	*M. gypseum*	12.5
		C.neoformance	100.0
Standard	Fluconazole	All tested fungi	0.025

CONCLUSION

The results validate the ethnobotanical use of the studied medicinal plants used among the Kipsigi community in Kenya. *Erithrina abyssinica* was very active on gram positive, gram negative bacteria and also fungal strains therefore it can be considered has having broad spectrum of activity. In this study, all the plant extracts had no activity against *E. coli* thus infections caused by this pathogen will not be treated by extracts from these plants.

RECOMMENDATIONS

It is worth recommending that since the plants extracts are potent both antibacterial and antifungal, further work should be done especially phytochemical 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. For safety it is worth recommending that *in-vitro* and *in-vivo* toxicity of the plant extracts and pure compounds be carried out. Since this study was based on the plants part used by herbalist, it is worth recommending that other parts be studied so as to give conclusive results. 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 in their natural environment so as to conserve our heritage.

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