

Antibacterial and cytotoxic activity of Kenyan medicinal plants

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Seven medicinal plant extracts traditionally used in Kenya, mainly for management of infectious conditions, were chosen and screened for their antibacterial activity against Gram-negative (Pseudomonas aeruginosa and Escherichia coli) and Gram-positive (Bacillus cereus and Staphylococcus aureus) bacteria. Antibacterial activity was tested using the broth dilution method. Harrisonia abyssinica and Terminalia kilimandscharica extracts showed significant activity against Gram+ and Gram- bacteria. The methanolic extracts of T. kilimandscharica bark and H. abyssinica bark and leaves showed minimum inhibitory activity against all tested bacteria, with minimal inhibitory concentrations ranging from 25-150 mg/mL. Ajuga remota and Amaranthus hybridus, which are lethal to brine shrimp nauplii, showed significantly lower antibacterial activity than those that were relatively non-toxic.

Key words: antibacterial - cytotoxicity - medicinal plants

Traditional medicine is widely practiced in Kenya, where this has been documented by ethnobotanical surveys (Miaron et al. 2004, Kareru et al. 2007, Njoroge & Bussman 2007). The high cost of imported conventional drugs and/or inaccessibility to western health care facilities has led to overreliance on traditional medicine since it is affordable and available to rural people. On the other hand, even when western health facilities are available, traditional medicine is viewed as an efficient and an acceptable system from a cultural perspective (Munguti 1997, Miaron et al. 2004).

Infections associated with bacterial pathogens are among some of the indications treated using traditional remedies in Kenya (Njoroge & Bussman 2007). Bacterial infections are prevalent due to various factors such as the HIV/AIDS pandemic, poor hygiene, overcrowding and resistance to conventional antimicrobials. Natural products of higher plants may provide a new source of antimicrobial agents with possibly novel mechanisms of action (Adenisa et al. 2000).

The aim of this study was to screen methanolic medicinal plant extracts traditionally used for the management of infectious diseases in Kenya. Brine shrimp (*Artemia salina* Leach) lethality test (BSLA) was used to systematically evaluate the cytotoxic effects of extracts. Broth dilution assays using Gram-negative bacteria *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) and Gram-positive bacteria *Staphylococcus aureus* (ATCC25923) and *Bacillus cereus* (ATCC 11778) was utilized to obtain the minimal inhibitory concentrations (MIC) of the plant extracts.

MATERIALS AND METHODS

Plant materials - Plants were collected during dry season from January-March 2006 in Machakos and Kitui areas of the Eastern province, Kenya, based on ethnopharmacological use through interviews with traditional health practitioners. The plants were identified by Mr. Ochung, a plant taxonomist, and voucher specimens (Table I) were deposited at the Department of Land Resource Management and Agricultural Technology Herbarium, University of Nairobi.

Extraction - The plant parts (whole plant, bark, root, leaves, tubers or a mixture) were chopped into small pieces, air dried at rt (25°C) under shade and pulverized using a laboratory mill yielding 300-400 g. The fine powders were separately soaked in 70% methanol (3 × 500 mL) for four days at rt to produce extracts. The extracts were filtered and concentrated using rota vapor at 50°C and 100 mbar. Further preparation was done according to Gebre-Mariam et al. (2006). The lyophilized extracts were kept at +4°C until they were screened. The yields of the methanol extracts were between 5%-8.4%. The methanolic extract yields were obtained as follows: leaves of *Ajuga remota* Benth (320 g) yielded 7.5%, leaves of *Amaranthus hybridus* L. (350 g) yielded 4.2%, leaves of *Cassia didymobotrya* Fres. (320 g) yielded 8.4%, a mixture of leaves and roots of *Croton macrostachyus* Del. (400 g) yielded 5.2%, tubers of *Entada leptostachya* Harms (300 g) yielded 6%, mixture of leaves and bark of *Harrisonia abyssinica* Oliv (360 g) yielded 6.5% and bark of *Terminalia kilimandscharica* Engl. (370 g) yielded 7.2%.

Antibacterial assay - Preliminary antimicrobial testing was done using the disk diffusion (Kirby-Bauer) method (Kirby et al. 1966). The disks were put in a clean glass bottle and sterilized at 121°C for 15 min in an autoclave. Broth dilution assay was used to screen the extracts for antibacterial activity. The bacterial strains were obtained from the Department of Public Health, Pharmacology and Toxicology, University of Nairobi.

Financial support: Commission of Higher Education (CHE/1/26/1/4, PI: JM Mbaria)

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Received 3 April 2008

Accepted 16 September 2008

TABLE I
Botanical identification, their uses, and parts used of medicinal plants studied

Species	Parts	Voucher n	Family	Vernacular name	Therapeutic indications
<i>Ajuga remota</i> Benth	L	CW5	Lamiaceae	Katetema	Malaria, boils
<i>Amaranthus hybridus</i> L.	Wp	CW10	Caesalpinaceae	Muvisi/musavula	Urinary tract infection, kidney and stomach ailments
<i>Cassia didymobotrya</i> Fres.	L	CW9	Caesalpinaceae	Muthaa	Typhoid
<i>Croton macrostachyus</i> Del.	L, R	CW6	Euphorbiaceae	Mukambi/kitundu	Typhoid, measles
<i>Entada leptostachya</i> Harms	T	CW4	Mimosaceae	Mwaittha	Tuberculosis, cough
<i>Harrisonia abyssinica</i> Oliv	L, B	CW1	Simaroubaceae	Muthiia	Pneumonia, syphilis, infertility, malaria, stomach, eye ointment
<i>Terminalia kilimandscharica</i> Engl.	B	CW3	Combretaceae	Muuku	Cough, sexually transmitted diseases

B: bark; L: leaves; R: roots; T: tuber; Wp: whole plant.

The bacterial inoculums of each reference bacteria were obtained from fresh colonies in Mueller Hinton agar (Oxoid® UK) plates. Suspension of approximately 2.2×10^8 cfu/mL in sterile normal saline was prepared according to Shahidi (2004). Stock solutions of plant extracts of 200 mg/mL were made separately for each plant species and, from this, two-fold serial dilutions were prepared in sterile Mueller Hinton Broth (Oxoid®, UK). A tenth of a milliliter of bacterial suspension was dispensed into each test tube. Pure solvents were used as negative controls. The tubes were incubated at 37°C for 24 h. The MIC was defined as the lowest concentration that inhibited any visible bacterial growth on the culture plates (Shahidi 2004). Benzyl penicillin and streptomycin antibiotics (Apothekernes Laboratorium, Oslo) were used as standard control drugs.

BSLA - Lethality testing was done according to McLaughlin (1991). Three dilutions of extract were prepared by transferring 500 µL, 50 µL and 5 µL to the set of five graduated tubes. Ten shrimp nauplii were transferred into each of the vial using Pasteur pipette. Marine salt was added to the 5 mL mark to produce a final concentration of 1000 µg/mL, 100 µg/mL and 10 µg/mL. After 24 h exposure, the live nauplii were counted and the LC₅₀ calculated using the probit analysis (Finney 1971). Standard controls with and without Vincristin (Oncovin®) were run simultaneously.

Ethics - Prior consent of respondents was obtained with adherence to National Regulatory guidelines on intellectual property rights. The field work surveys and quantitative data collection were conducted in accordance with the institutional, national and international principles and guidelines of plant use and conservation of biodiversity.

RESULTS AND DISCUSSION

Results are shown in Tables 1-3. The antibacterial activity was considered significant if the MIC \leq 200 mg/mL (Suffredini et al. 2006). The extracts of *T. kilimandscharica* and *H. abyssinica* produced significant activity against all test bacteria. *B. cereus* was inhibited at an MIC of \leq 150 mg/mL by all the plant extracts screened in the current study. From the seven species studied, 28% showed inhibitory activity against *Ps. aeruginosa*

at \leq 75 mg/mL. The extract of bark and mixture of bark and leaves were the most active against the test bacteria. The tuber extract of *E. leptostachya* was the least active; *Ps. aeruginosa* showed resistance while *B. cereus* was the most sensitive to plant extracts.

Brine shrimp is a low cost bench bioassay indicative of cytotoxic, pesticidal, heavy metal toxicity, insecticidal and antifeedant properties. There was no correlation between BSLA and bacteriostatic properties that could be deduced in this study. Nonetheless, the plant extracts that were lethal to brine shrimp nauplii also had low inhibitory activity against bacteria. From the plant extracts, only *A. remota* had an LC₅₀ of $<$ 100 µg/mL, which was considered toxic. The other plant species had an LC₅₀ of $<$ 1000 µg/mL, which was considered relatively non-toxic (Santos et al. 2003).

Some of the genera investigated in the present study have been known to contain pharmacologically active compounds. Combrestatins and flavonoids have been isolated from *Terminalia* sp. (Garcez et al. 2006), terpenoids, glycosides, ajugarin I and II have been isolated from the *Lamiaceae* family (Matu & van Staden 2003, Manguro et al. 2006), antioxidants and phenols from *A. remota* (Chitindingu et al. 2007), an antifungal thaumatin-like protein from *Cassia didymobotrya* (Vitali et al. 2006) and saponins isolated from *E. leptostachya* (Ka-reru et al. 2008).

The present study supports the continued sustainable use of medicinal plant resources backed by scientific validation. Phytochemical elucidation of antibacterial and cytotoxic principles of *H. abyssinica*, *A. remota*, *A. hybridus*, *T. kilimandscharica* and *C. didymobotrya* should be undertaken with the objective to isolate the active biochemical principles and develop novel antimicrobial agents.

ACKNOWLEDGEMENTS

To members of Ukamba Herbalist Society, for their cooperation during the field data collection, and to Mr. Ochung, Herbarium Curator, LARMAT, University of Nairobi, for botanical identification of the plants.

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TABLE II
Minimum inhibitory concentration obtained for methanolic extracts

Plant species	Bacterial strains (mg/mL)			
	<i>S. aureus</i>	<i>B. cereus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
<i>Ajuga remota</i> Benth	7.8	15.6	-	31.25
<i>Amaranthus hybridus</i> L.	7.8	15.6	-	31.25
<i>Cassia didymobotrya</i> Fes	15.6	15.6	-	62.5
<i>Croton macrostachyus</i> Del.	-	15.6	250	250
<i>Entada leptostachya</i> Harms	-	125	250	-
<i>Harrisonia abyssinica</i> Oliv	25	15.6	37.5	150
<i>Terminalia kilimandscharica</i> Engl.	100	15.6	75	150
Streptomycin ^a			0.25	0.25
Benzylpenicillin ^a	0.6	0.6		

a: antibiotics.

TABLE III
Brine shrimp test of methanol extracts. The results are presented as lethal concentration 50 (LC₅₀) values (µg/mL) and 95% confidence

Botanical name	Parts	LC ₅₀ (µg/mL)	95% CI
<i>Ajuga remota</i> Benth.	L	61.6	19.8 - 144.6
<i>Amaranthus hybridus</i> L.	Wp	116.1	45.1 - 316.7
<i>Cassia didymobotrya</i> Fres.	L	230.9	91.3 - 650.9
<i>Croton macrostachyus</i> Del.	L, R	387.1	160.2 - 936.9
<i>Entada leptostachya</i> Harms.	T	421.3	127.9 - 6301.3
<i>Harrisonia abyssinica</i> Oliv.	L, B	392.4	103.1 - 15928.6
<i>Terminalia kilimandscharica</i> Engl.	B	164.1	56.6 - 502.8
Vincristri (Oncovin®)		< 1	

B: bark; CI: intervals; L: leaves; R: roots; T: tuber; Wp: whole plant.

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