PHYTOTHERAPY RESEARCH
Phytother. Res. (2009)
Published online in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/ptr.2866

SHORT COMMUNICATION

Screening of some Kenyan Medicinal Plants for Antibacterial Activity

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Eleven medicinal plants used by traditional healers in Machakos and Kitui District were screened, namely: *Ajuga remota* Benth, *Aloe secundiflora* Engl, *Amaranthus hybridus* L, *Cassia didymobotrya* Fes, *Croton macrostachyus* Del, *Entada leptostachya* Harms, *Erythrina abyssinica* DC, *Harrisonia abyssinica* Oliv, *Schkuhria pinnata* O. Ktze, *Terminalia kilimandscharica* Engl and *Ziziphus abyssinica* Hochst for potential antibacterial activity against four medically important bacterial strains, namely: *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 25922, *Micrococcus lutea* ATCC 9341 and *Pseudomonas aeruginosa* ATCC 27853. The antibacterial activity of methanol extracts was determined as the minimum inhibitory concentration (MIC). The plant extracts were more active against Gram-positive (G+) than Gram-negative (G–) bacteria. The positive controls were streptomycin and benzylpenicillin for G– and G+ bacteria, respectively, both had a significant MIC at <1 mg/mL. The most susceptible bacteria were *B. cereus*, followed by *M. lutea*, while the most resistant bacteria were *Ps. aeruginosa*, followed by *E. coli*. The present study supports the use of these plants by the herbalists in the management of bacterial ailments. *H. abyssinica* and *T. kilimandscharica* showed the best antibacterial activity; hence these plants can be further subjected to phytochemical and pharmacological evaluation. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: medicinal plants; antibacterial activity; minimum inhibitory concentration.

INTRODUCTION

Plants form an integral part of life in many indigenous African communities as a readily and cheaply available alternative to allopathic medicines. Due to either limited availability or affordability of pharmaceutical medicines about 80% of the rural population in Sub-Saharan Africa (SSA) depends on traditional herbal remedies for primary health care (PHC) and veterinary use (WHO, 2002).

Multiple drug resistance (MDR) has developed due to the indiscriminate use of antimicrobials and re-emergence of diseases; adverse drug reactions (ADR) and the high costs of antimicrobials have been key contributors to ineffective management of infectious diseases in many developing countries (Kapila, 2005; Runyoro *et al.*, 2006). Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action (Runyoro *et al.*, 2006; Shahidi, 2004).

Poverty, resistance to antiretroviral drugs (ARV) and opportunistic infections are the main drawbacks to the management of HIV/AIDS in Africa necessitating venturing into alternative traditional medicines (Kisangau *et al.*, 2007).

The provision of safe and effective traditional medicines could become a critical tool to increase access to health care (WHO, 2002). In the past few decades there have been intense pharmacological studies, brought about by the recognition of the value of medicinal plants as potential sources of new compounds for therapeutic use. The objective of this study was to test the antimicrobial activity of different plant extracts used by traditional healers in Machakos and Kitui Districts, Kenya.

MATERIALS AND METHODS

Plant material. The 12 plant samples (Table 1) screened in this study were collected in January–March 2006 from Machakos and Kitui Districts of Kenya (Fig. 1) based on ethnopharmacological use through interviews with traditional health practitioners (THP). Information gathered included vernacular names and the parts used in preparation of herbal antibacterial remedies. The plants were identified by Mr Ochung (Department of Land Resource Management and Agricultural Technology (LARMAT), University of Nairobi). Voucher specimens were deposited at LARMAT Herbarium.

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Received 28 March 2008
Accepted 13 March 2009

Table 1. Botanical identification, their uses and parts used of selected medicinal plants studied

Species, (Parts), voucher no.	Family	Vern.	Therapeutic indications
Ajuga remota Benth, (L), CW5	*Lamiaceae*	Katetema	Malaria, boils
Aloe secundiflora Engl, (Wp), CW8	*Aloaceae*	Kiluma	Malaria, pneumonia
Amaranthus hybridus L, (Wp), CW10	*Caesalpinaceae*	Muvisi/musavula	Urinay tract infection (UTI), kidney and stomach ailments
Cassia didymobotrya Fes, (L), CW9	*Caesalpinaceae*	Muthaa	Typhoid
Croton macrostachyus Del., (L,R), CW6	*Euphorbiaceae*	Mukambi/kitundu	Typhoid, measles
Entada leptostachya Harms, (T), CW4	*Mimosaceae*	Mwaitha	Tuberculosis, cough
Erythrina abyssinica DC., (B,R), CW2	*Pappilionaceae*	Kivuti	Pneumonia, STDs, prostate
Harrisonia abyssinica Oliv, (L,B), CW1	*Simaroubaceae*	Muthiia	Pneumonia, syphilis, infertility, malaria, stomach, eye ointment
Schkuhria pinnata O. Ktze ,(Wp), CW12	*Compositae*	Kaututi	Malaria, joint pains, diabetes
Terminalia kilimandscharica Engl., (B), CW3	*Combretaceae*	Muuku	Cough, sexually transmitted diseases (STD)
Ziziphus abyssinica Hochst, (B,R), CW7	*Rhamnaceae*	Kiae	Kidney, stomach

Parts used: L, leaves; Wp, whole plant; R, roots; B, bark; T, tuber; Vern, vernacular name.

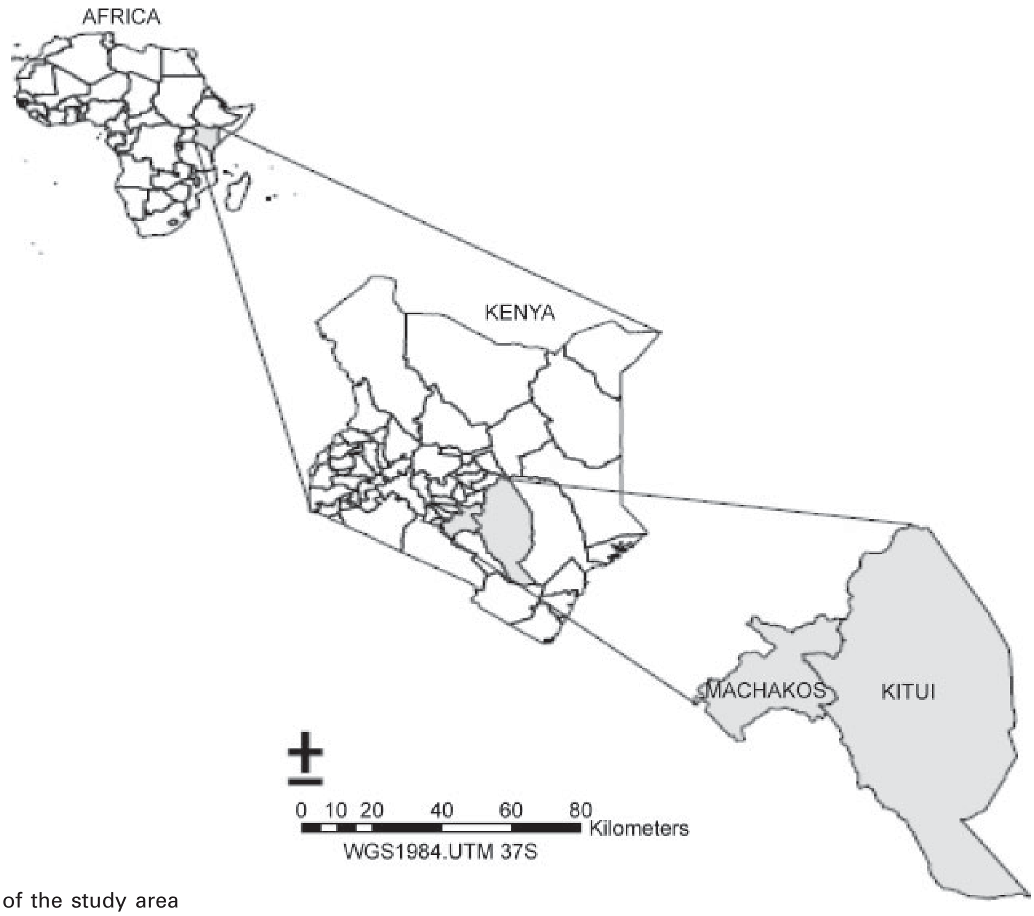

Figure 1. Map of the study area

Preparation of plant extracts. The plant parts (whole plant, bark, root, leaves, tubers or a mixture) were chopped into small pieces, air dried at room temperature (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 4 days at room temperature to produce extracts. The extracts were filtered and concentrated using a rota-vapour at 50 °C and 100 mbar. Further preparation was done according to Gebre-Mariam *et al.* (2006). The yields of the methanol extracts were between 1.6% and 8.4%.

Test microorganisms. To test for antibacterial activities of the methanol extracts, the bacterial reference strains, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Micrococcus lutea* (ATCC 9341) and *Bacillus cereus* (ATCC 11778) obtained from Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nairobi, Kenya were used.

Evaluation of antibacterial activity. The broth dilution method was used to determine the minimum inhibitory concentration (MIC) of each plant extract against the reference bacterial strains. The bacterial strains were cultured overnight at 37 °C in blood agar (Oxoid® UK). The test strains were suspended in sterile physiological saline to give a final density of 2.2×10^8 cfu/mL. Serial two-fold dilution of plant extracts was made in Muller Hinton broth (Oxoid® UK). Using sterile a 1 mL pipette, 0.1 mL of bacterial suspension was dispensed into each of the test tubes. The last tube containing Muller Hinton broth (Oxoid® UK) without extract and 0.1 mL of the inocula was used as a negative control. Standard antibiotics benzylpenicillin and streptomycin (Apothekernes Laboratorium, Oslo) were used as positive controls for Gram-positive and Gram-negative bacteria, respectively. The MIC was defined as the lowest concentration that inhibited any visible bacterial growth on the culture plates (Shahidi, 2004).

RESULTS AND DISCUSSION

Table 1 lists the plants studied. The minimum inhibitory concentration is represented in Table 2.

All plant extracts showed activity against at least one organism except *Erythrina abyssinica*. *B. cereus* was the most sensitive bacterium to the extracts with 62.2% of plant extracts showing *in vitro* activity against *B. cereus* at concentrations in the range 7.8–150 mg/mL. *Pseudomonas aeruginosa* was least sensitive with only 45% of plant extracts having inhibitory activity. The lack of sensitivity of *Ps. aeruginosa* could be attributed to the fact that the bacteria is naturally resistant to many antibiotics due to the permeability barrier offered by its outer membrane.

The most active plant extracts against *Ps. aeruginosa* were *Amaranthus hybridus* and *Ajuga remota* at 31.25 mg/mL. *Harrisonia abyssinica* extract showed activity at lower concentrations in the range 15.6–150 mg/mL. Compared with other plant extracts *A. remota* and *A. hybridus* had the lowest MIC in the range 7.8–31.25 mg/mL.

E. coli was not sensitive to extracts of *Schkuhria pinnata*, *Ziziphus abyssinica*, *Erythrina abyssinica* and *Harrisonia abyssinica*. The other extracts had MIC in the range 31.25–250 mg/mL with *A. remota* and *A. hybridus* having the lowest MIC of 31.25 mg/mL. The extracts of *Terminalia kilimandscharica* showed a broad spectrum of activity against all bacterial strains at tested concentrations of 15.6–150 mg/mL.

According to Suffredini *et al.* (2006), Gram-negative bacteria are hardly susceptible to plant extracts in doses as low as 200 mg/mL, thus neat concentrations of 250 mg/mL were made and this was used in testing the antibacterial activity for Gram-negative bacteria. Due the multi-resistance nature of *P. aeruginosa*, plants such as *H. abyssinica* and *T. kilimandscharica* are promising candidates in the search for novel antibacterial principles. *H. abyssinica* was indicated by the herbalists for an array of conditions.

A. hybridus has great potential for the treatment of coliform urinary tract infection as indicated by the current study. Chitindingu *et al.* (2007) isolated antioxidant and phenolic substances from *A. hybridus* which are known to have anti-human immunodeficiency virus (HIV) properties. Thus the use of this leafy vegetable is recommended for combating opportunistic related infections in HIV/AIDS patients.

Matu and van Staden (2003) reported the absence of antibacterial activity of methanol leaf extracts of *C. macrostachyus*. In this study, however, the root and leaf extracts were active against *B. cereus*, *Ps. aeruginosa* and *E. coli*. This contrasting observation could be attributed to the locality of plant species, parts used, time of collection, storage conditions and methods of analysis.

Previous studies by Matu and van Staden (2003) reported that extracts of *A. remota* had no antibacterial activity on the test bacteria. The current study shows otherwise, the family *Lamiaceae* contains terpenoids which are known to possess antifungal, antibacterial and antipesticidal activities.

Previous studies have reported the antibacterial properties of *A. secundiflora* (Waihenya *et al.*, 2002) and the current study corroborates this. The extract of *E. leptostachya* showed appreciable inhibitory activity and its use for tuberculosis management as reported by herbalists should be studied in detail using appropriate methodologies.

Table 2. *In vitro* antibacterial activity (MIC) of methanol extracts after incubation of serial dilution (mg/mL) of selected medicinal plants

	Reference bacterial strain			
Plant specimen	*M. lutea*	*B. cereus*	*Ps. aeruginosa*	*E. coli*
Ajuga remota Benth	7.8	15.6	–	31.25
Aloe secundiflora Engl.	–	7.8	250	250
Amaranthus hybridus L.	7.8	15.6	–	31.25
Cassia didymobotrya Fes	15.6	15.6	–	62.5
Croton macrostachyus Del.	–	15.6	250	250
Entada leptostachya Harms	–	125	250	–
Erythrina abyssinica DC.	–	–	–	–
Harrisonia abyssinica Oliv	25	15.6	37.5	150
Schkuhria pinnata O. Ktze	100	–	–	–
Terminalia kilimandscharica Engl.	100	15.6	75	150
Ziziphus abyssinica Hochst	–	28.75	–	–
Streptomycin[a]			0.25	0.25
Benzylpenicillin[a]	0.6	0.6		

[a] Antibiotics.

DOI: 10.1002/ptr

The antibacterial activity was both dose and bacterial strain dependent. The action of the methanol extract was at a higher inhibitory concentration than that of the commercial antimicrobial used as positive controls.

In conclusion, the results of this study support the documented use of these plants and others in the management of infectious diseases in this study area and elsewhere. Further phytochemical analyses to elucidate the active pharmacological principles and toxicological evaluation of the medicinal plants are needed.

Acknowledgements

We are grateful to members of Ukamba Herbalist Society (UHS) for their cooperation during the field data collection. The authors are grateful to Mr Ochung, Herbarium Curator, LARMAT, University of Nairobi for botanical identification of the plants.

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DOI: 10.1002/ptr