Antimicrobial and brine shrimp toxicity of some plants used in traditional medicine in Bukoba District, north-western Tanzania

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Abstract: Herbal medicines constitute a potentially important resource for new and safe drugs for the management of microbial infections and other diseases. In this study, dichloromethane, ethylacetate and ethanol extracts of *Canarium schweinfurthii* Engl., *Dissotis brazzae* Cong., *Iboza urticifolia* (Bak) E.A.Bruce, *Isoglosa lacteal* Lindau, *Strombosia Scheffleri* Engl., and *Whitfieldia elongate* T. Anders were tested for antimicrobial activity and brine shrimp toxicity. The objective was to validate claims that they are used to treat bacterial infections, diarrhoea and heal wounds among the Haya tribe of north-western Tanzania. At least one extract of each plant showed antibacterial activity. Dichloromethane extracts were the most active while ethanol extracts were the least active. Extracts of *Whitfieldia elongate* and *Isoglossa lacteal* were the most and least active with MICs in the range 0.08-0.62 mg/ml and 15.6-62.5 mg/ml, respectively. The dichloromethane extract of *Whitfieldia elongate* exhibited strong antifungal activity against *Cryptococcus neoformans.* Against brine shrimp larvae, the extracts from the six plants exhibited a low to very low toxicity with LC_{50} values ranging from 15.35-374.0µg/ml. However, ethanol extracts of *Dissotis brazzae* and *Strombosia scheffleri* had LC_{50} values of >1000µg/ml. The seemingly innocuous nature and relatively good antibacterial activity against skin infections and gastrointestinal pathogenic bacteria support the traditional uses of the plants and deserve more detailed studies.

Keywords: antimicrobials, brine shrimp toxicity, traditional medicines, Tanzania

Introduction

Plants used traditionally as medicines constitute a potentially useful resource for new and safe drugs for the treatment of microbial infections and other diseases. The Kagera Region of north-western Tanzania is one of the places where traditional medicines are widely used, and play a significant role in the provision of health care. According to *Medicine du Monde*, a French non-governmental organization in Kagera Region, five out of every six HIV patients receive their medical attention from a traditional healer rather than from a hospital or primary health care facility (AIDS Analysis, 1996). This is not unique for Kagera Region or rural areas only as indicated by a survey conducted in Dar es Salaam which showed that 21% of the people who seek care from public services had first consulted a traditional healer (Kilima *et al*., 2003).

The purpose of the present study is to validate claims on six plants that are used by traditional healers in Bukoba Rural District of Kagera Region in north-western Tanzania for the management of bacterial and fungal infections and for wound healing. The brine shrimp lethality test was used as a surrogate tool to evaluate their toxicities, and also to identify their potential for other biological activities.

Materials and Methods

Study site

The plants used in this study were collected from Buzi village in Bugabo Ward in Bukoba Rural District, north-western Tanzania. Duplicate vouchers are kept at the Herbaria of the Botany Department University of Dar es Salaam and that of the Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences. The voucher numbers are listed in Table 1.

Materials

Dimethyl sulphoxide (DMSO) was purchased from Sigma (Poole, Dorset, UK) and ethanol, dichloromethane and ethyl acetate from Fisher Scientific UK Ltd (Bishop Meadow Road, Loughborough, Leicestershire, LE 11 5RG, UK). Saboraud's dextrose agar (SDA) and Tryptone soya agar and broth were purchased from Oxoid Ltd (Basingstoke, Hampshire, England), while Iodonitro tetrazolium chloride was

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purchased from Sigma (Poole, Dorset, England). Brine shrimp eggs were bought from Dohse Acquaristic, Bonn (Aus Dem Hause Dohse Acquaristik), Germany. Sea salt was prepared locally by evaporating water collected from the Indian Ocean, along the Dar es Salaam coast.

Preparation of plant extracts

The plant materials were ground into powders and sequentially soaked for 48h, with dichloromethane, ethyl acetate and ethanol. The extracts were dried using a rotary evaporator, at 40^{O}C, followed by freeze drying to remove any remaining water. The powders were then stored at -20^{O} C until the time of testing.

Antimicrobial tests

The plant extracts were all dissolved in dimethyl suphoxide. Antibacterial and antifungal activities were tested by the disc-diffusion method (Singh et al., 2002). Eight standard bacteria, *Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (NCTC 10418), *Pseudomonas aeruginosa* (NCTC 10662), Salmonella *typhi* (NCTC 8385), *Bacillus cereus* (NCTC 7464), *Proteus mirabilis* (NCTC 10975), *Shigella flexineri* (clinical isolate), *Vibrio cholerae* (clinical isolate) and two fungi, *Candida albicans* (Strain HG 392), *Cryptococcus neoformans* (clinical isolate) were used. Filter paper discs (Whatman No. 1; 5 mm diameter) were impregnated with crude extracts (5 mg/disc) or standard drugs (10 µg/disc gentamicin; for bacteria) and clotrimazole (20 µg/disc for fungi). The discs were overlaid on pre-inoculated tryptone soya agar plates (for bacteria) and Saborauld's dextrose agar plates (for fungi) and incubated at 37 ^{O}C, for 24 h. The discs were tested in triplicate, including one with a solvent blank and three with the standard drugs. The results of the disc diffusion method were only used to detect active extracts for minimum inhibitory concentrations (MIC) determination using the microdilution method. This was necessary to cut down on wastage of the microtitre plates.

Determination of MICs

MICs were determined using the microdilution method (Ellof, 1998). The 96 well microtitre plates were used and each plant extract was tested in duplicate at serial dilutions of 0.08, 0.16, 0.31, 0.625, 1.25, 2.5, 5 and10 mg/ml. Columns 1 and 2 were used for solvent controls and Columns 11 and 12 for positive controls. Each well was filled with 100µl broth, 100µl test drug, and 100 µl inoculated broth and incubated overnight at 37^{0}C. Two hours before reading the results, 40µl of the indicator (0.2% nitrotetrazolium chloride were added to the plates and incubation continued for a maximum of two hours. At this point the plates were inspected for colour change; blue coloration indicating presence of microbial growth. MICs were recorded as the last well in a row where there was no colour change.

Brine shrimp lethality test

The brine shrimp lethality test (BST) was used to predict the PResence, in the extracts, of cytotoxic activity (Meyer *et al*., 1982). Solutions of the extracts were made in DMSO or distilled water, at varying concentrations and incubated in duplicate vials with the brine shrimp larvae. Ten brine shrimp larvae were placed in each of the Duplicate vials. Control brine shrimp larvae were placed in a mixture of sea water and DMSO only. After 24h the vials were examined against a lighted background and the average number of larvae that survived in each vial was determined. The mean percentage mortality was plotted against the logarithm of concentrations and the concentration killing fifty percent of the larvae (LC_{50}) was determined from the graph (Meyer *et al*., 1982).

Data analysis

The mean results of brine shrimp mortality against the logarithms of concentrations were plotted using the Fig P computer program (Biosoft Inc, USA), which also gives the regression equations. The regression equations were used to calculate LC_{16}, LC_{50} and LC_{84} values. Confidence intervals (95% CI) were then calculated using the three results (Litchfield & Wilcoxon, 1949; Meyer *et al*., 1982). A LC_{50} value greater than 100µg/ml was considered to represent an inactive compound or extract

Results

Antimicrobial tests

Available information of the six of the plants that are used among the Haya in Bukoba Rural District to treat bacterial infections and for healing wounds are summarized in Table 1. The ethnomedical claims on antimicrobial or wound healing functions are reported here for the first time. The MIC results are summarized in Table 2 and indicate that some plants had strong antibacterial activity.

Table 1: Ethnobotanical information of plants collected from Buzi Village, Bukoba District

Botanical name	Vernacular name (Voucher No.)	Conditions treated, part used and preparation	Literature reports
Canarium schweinfurthii Engl. (BURSERACEAE)	Muubani (3036)	Malaria and syphilis. Stem bark ground and boiled with water	Bark used to treat gonorrhoea, worms and as stomachic (Gill & Akinwami, 1986), syphilis (Kamdem *et al*., 1986), diarrhoea (Noumi & Yomi, 2001), diabetes (Kamtchouing *et al*., 2006). Water extract of bark is parasympatholytic (Sandberg & Cronlund, 1977)
Dissotis brazzae Cong (MELASTOMACEAE)	Katuntunu (3098)	Syphilis and malaria. Leaves are boiled together with those of *Solanum nigrum* L. (Solanaceae)	Decoction of the leaf is used to induce sleep and has antihelmintic activity (Chhabra & Mahunnah, 1994). Entire plant is galactagogue (Lewalle & Rodegem, 1968). Different extract of the plant have antimalarial activity (Omulokoli *et al*., 1997).
Iboza urticifolia (Bak) E.A. Bruce (LABIATAE)	Mkono wa nkhanda/ekiho/ kiswija (3125)	Ulcers, tonsils, wounds and malaria. A decoction is made and drank or leaves are pounded and used for dressing wounds.	No reported ethnomedical use or biological activity
Isoglosa lacteal Lindau (Acanthaceae)	Omufoka (3085)	Discolouration of the skin (loss of melanin), syphilis and other conditions. Aerial parts boiled and the decoction drank or applied topically.	No reported ethnomedical use or biological activity
Strombosia scheffleri Engl.(Olaceae)	Mmarara (3032)	Treatment of diarrhoea/hydrofoetalis. Stem bark and leaves boiled with water and decoction drank	No reported ethnomedical use or biological activity
Whitfieldia elongate T. Anders (Acanthaceae)	Ekigenge (3062)	Chicken pox and other skin conditions and rectal prolapse. Leaves are squeezed and exudates taken orally/ Exudates can be applied topically	No reported ethnomedical use or biological activity

Using the disc diffusion method, the dichloromethane, ethylacetate and ethanol extracts from *Dissotis brazzae* were active against *B. cereus, V.cholerae, S. typhi, and S. flexineri*. The most active of the extracts was dichloromethane followed by ethyl acetate and ethanol respectively. All the extracts were inactive against the two fungi used. Extracts of *Isogolossa lacteal* exhibited only antibacterial but no antifungal activity. Both the dichloromethane and ethyl acetate extracts exhibited weak activity against *V. cholerae* and *S. aureus* and in addition, the ethyl acetate extract also showed weak activity against, *P. aeruginosa* and *E. coli*. The dichloromethane extract of *Whitfieldia elongate* showed strong activity against *V. cholerae, S. aureus, B. cereus,* and *C. neoformans* while the ethylacetate extract was active against *V. cholerae, S. aureus,* and *B. cereus*. The ethanol extract, however, exhibited weak activity against the same bacteria. Due to shortage of extracts, MICs were determined only for dichloromethane and ethylacetate extracts (Table 2)

Table 2: Antimicrobial activity expressed as Minimum inhibitory concentrations (MICs) in mg/ml

Plant name	Organism tested	MIC values of extracts (n=2)		
		Dichloromethane	Ethylacetate	Ethanol
Dissotis brazzae	*Staphylococcus aureus*	1.95	3.91	-
	Bacillus cereus	0.24	0.49	7.81
	Vibrio cholera	0.98	0.98	7.81
Isoglossa lactael	*Bacillus cereus*	15.6	15.6	-
	V. cholerae	7.8	31.2	-
	P. aeruginosa	-	62.5	-
	S. aureus	7.8	31.2	-
Whitfieldia Elongate	*V. cholerae*	0.16	0.62	
	S. aureus	0.62	-	
	C. neoformans	0.08		
Iboza urticifolia	*S. aureus*	-	10	-
	B. cereus	2.5	2.5	-
Strombosia scheffleri	*V. cholerae*	0.62	-	
Canarium Schweinfurthii	*V. cholerae*	0.62	-	0.62
	S. aureus	-	10	-
	P. vulgaris	-	5	10

The dichloromethane extract of *Iboza urticifolia* showed activity against *S. aureus* while the ethyl acetate extract was only active against *B. cereus.* The ethanol extract was active against *S. aureus* and *B. cereus.* The dichloromethane extract of *Strombosia scheffleri* was active against *V. cholerae, S. aureus* and *P. aeruginosa* and the ethanol one against *P. aeruginosa.* There was no enough extract to do MICs for all the available organisms. The dichloromethane extract of *Canarium schweinfurthii* showed activity against *V. cholerae,* while the ethylacetate extract was active against *S. aureus* and *P. vulgaris*. The ethanol extract was active against *V. cholerae* and *P. vulgaris.*

Brine shrimp toxicity

The LC_{50} results range from 15.35-336.72 µg/ml for all extracts of the six plants, except the ethanol extracts of *Dissotis brazzae* and *Strombosia scheffleri* which gave LC_{50} values >1000 µg/ml (Table 3). Cyclophosphamide, which was used as a positive control gave an LC_{50} of 16.33 (10.60-25.15) µg/ml.

diseases and diarrhoea. The study is premised on establishing proof of traditional claims for antimicrobial activity, but also to roughly ascertain their safety. All the organisms used have clinical relevance related to either skin infections, diarrhoeal diseases or opportunistic fungal infections. Of the six plants used in Bukoba, only *Canarium schweinfurthii* is reported in the literature as being used for treating infection related conditions (Gill & Akinwumi, 1986; Noumi & Yomi, 2001). The ethnomedical claims on antimicrobial or wound healing functions are reported here for the first time.

Antimicrobial tests indicate that all the six plants have antibacterial activity against one or more pathogenic bacteria. Generally for all the six plants dichloromethane extracts were the most active against bacteria and for *Whitfieldia elongate*, it was the only extract active against *Cryptococcus neoformans*. There are no validated criteria for the MIC end points for *in vitro* testing of plant extracts, but a criterion

Table 3: Brine shrimp toxicity expressed as LC_{50} µg/ml (95% Confidence Intervals)

Plant name	LC_{50} µg/ml		
	Dichloromethane	Ethylacetate	Ethanol
Dissotis brazzae	64.21(45.79-90.04)	75.74 (69.34-82.74)	>1000
Isogolossa lactae	36.2 (27.0-48.6)	37.4 (26.2-53.4)	137.4 (86.3-218.6)
Whitfieldia elongate	15.35 (8.25 - 28.29)	54.16 (39.91 - 73.49)	62.36 (35.90 - 82.2)
Iboza urticifolia	22.43 (7.77 - 64.78)	18.77 (11.48 - 30.68)	36.48 (15.33 - 62.99)
Strombosia scheffleri	374.01(196.8 - 710.60)	132.17 (87.89 -198.76)	>1000
Canarium schweinfurthii	106.93 (73.01 - 156.60)	29.95 (18.92 - 47.38)	10.67 (5.81 - 19.41)
Cyclophosphamide	16.33 (10.60-25.15)		

Discussion

The plants used in this study were chosen on the basis that they are used traditionally for treatment of conditions such as skin infections, dressing of wounds, treatment of venereal

based on MIC results was proposed as follows: Strong inhibitors – MIC up to 0.5 mg/ml; moderate inhibitors – MIC between 0.6 and 1.5 mg/ml and weak inhibitors – MIC above 1.6 mg/ml (Aligiannis *et al*., 2001). According to this classification dichloromethane extracts

of *Strombosia scheffleri, Canarium schweinfurthii, Whitfieldia elongate* and *Dissotis brazzae* showed moderate to strong antibacterial activity against one or more of the bacteria used. *Whitfieldia elongate* was the only species in which the dichloromethane extract exhibited strong antifungal activity. These results, therefore, provide evidence of the efficacy of extracts from these plants to treat bacterial and fungal infections. Although the activities may not be very strong for some of the extracts, it is worth noting that some of the plants are used in combination to enhance their activity. For example *Dissotis brazzae* is used in combination with *Solanum nigrum* L. (Solanaceae).

The brine shrimp toxicity results suggest that the extracts of the six plants are fairly selective for infective organisms as they did not show high toxicity against the brine shrimps. Intuitively, it is therefore tempting to suggest that their short term use may not cause serious side effects. The extracts from the six plants used in this study provide promising leads for the isolation of antimicrobial compounds, and are therefore worth investigating further using bioassay guided fractionation.

In conclusion the therapeutic claims on the six plants used as traditional anti-infectives have been supported by the results which show positive activity of the extracts against skin and gastrointestinal pathogenic bacteria. However, further studies should be done to determine the real potential for their clinical application.

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References

AIDS Analysis Africa, February 1996, pp 12-13.

Aligiannis, N., Kalpotzakis, E., Mitaku, S. & Chinou, I.B (2001). Composition and antimicrobial activity of the essential oils of two *Origanum* species. *Journal of Agricultural and Food Chemistry* 40,4168–4170.

Chhabra, S.C. & Mahunnah,R.L.A. (1994) Plants using traditional medicine by Hayas of the Kagera Region, Tanzania. *Economic Botany* 48,121-129.

Ellof, J.N. (1998) A quick microplate method to determine the minimal inhibitory sensitive and concentration of plant extracts for bacteria. *Planta Medica* 64,711-713.

Gill,L.S. & Akinwumi, C. (1986) Nigerian folk medicine: practices and beliefs of the Ondo people. *Journal of Ethnopharmacology* 18, 259-266.

Kamdem, L., Messi, H.M., Ndongo, N.A., Mbi, C., Njikam, A.P. & Elobo,S. (1986) Ethnobotanical investigations carried out in Mouloundou (Eastern Province) and Zoetele (Southern Province). *Rev Sci Technol (Health Science Series)* 1986, 3, 59-68.

Kamtchouing, P., Kahpui, S.M., Dzeufiet, P.D.D., Tedong, L., Asongalem, E.A. & Dimo, T. (2006) Anti-diabetic activity of methanol/methylene chloride stem bark extracts of *Terminalia superba* and *Canarium schweinfurthii* on streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology* 104, 306-309.

Kilima, P.M., Ostermayer, I., Shija, M., Wolff, M.M. & Evans, P.J. (1993) Drug utilization, prescribing habits and patients in City Council Health Facilities, Dar es Salaam, Tanzania. *DUHP, Swiss Tropical Institute*, Basel, p. 19.

Lewalle, J. & Rodegem, F.M.(1969) Medicinal plants of Burundi. *Quarterly Journal of Crude Drug Research* 8, 1257.

Litchfield, Jr. J.R. & Wilcoxon, F. (1949) A simplified method of evaluating dose-effect experiments. *Journal of Pharmacology and Experimental Therapeutics* 96, 99-138.

Meyer, B.N., Ferrign, R.N., Putnam, J.E., Jacobson, L.B., Nicholas, D.E. & McLaughlin, J.L. (1982) Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Medica* 45, 31-34.

Noumi, E. & Yomi, A. (2001) Medicinal plants used for intestinal diseases in Mbalmayo Region, Central Province, Cameroon. *Fitoterapia* 72,246-254

Omulokoli, E., Khan, B. & Chhabra, S.C. (1997) Antiplasmodial activity of four Kenyan medicinal plants. *Journal of Ethnopharmacology* 56,133-137.

Sandberg, F. & Cronlund, A. (1977) What can we still learn from traditional folklore medicine? examples from the results of a biological screening of medicinal plants from equatorial Africa. *Proceedings of the 3rd Asian Symposium Medicinal Plants & Spices*, Colombo, Sri Lanka, 3,178-197.

Singh, B., Sahu, P.M. & Sharma, M.K. (2002) Anti-inflammatory and Antimicrobial activities of triterpenoids from *Strobilanthes callosus* Nees. *Phytomedicine* 9, 355-359.