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Cytotoxic Screening of Some Tanzania Medicinal plants

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Twenty plants that are used in traditional medicine in Iringa, Tanzania, were tested for *in vitro* cytotoxic activity on human bladder carcinoma (RT-4), colon adenocarcinoma (HT29), and skin carcinoma (A431) cell lines. At 100 µg/ml *Albizia harveyi, Albizia anthelmintica, Dalbergia nitidula, Euphorbia grantii* and *Rauvolfia caffra* reduced cell proliferation to 50% or more of the three cell lines. *Albizia harveyi* showed a significant cytotoxic activity on the RT-4 cell line (percentage survival 23%) at 10µg/ml. It showed a weak cytotoxic activity on the HT-29 cell line. *Dalbergia nitidula* showed a weak cytotoxic activity with percentage death of the RT-4 and HT-29 cell lines of 39 and 34%, respectively, at the 10 µg/ml level. These results show that 19 (95%) of the plant extracts tested are non-toxic. One plant (5%), *Albizia harveyi* showed cytotoxic activity on one of the cell lines used, which was in agreement with the accepted detection level of biological activity by chance. Bioassay guided fractionation of the plant extracts to identify active compound(s) is suggested.

Keywords: Cytotoxicity, human cancer cell lines, plant extracts

INTRODUCTION

Tanzania has a wealth of flora comprising over 10,000 plant species, of which 1122 are endemic [1,2]. Many of these plants are used as medicines by the rural populations [3]. It has also been reported that 21% of the people who utilize public health services in Dar es Salaam consult a traditional healer before doing so [4]. This finding indicates the popularity of traditional medicines and point to the possibility that some of these plants may indeed be effective, although most of them are yet to be exploited.

One of the ways in which these therapies could be identified and utilized is by conducting disease-related ethnobiomedical surveys. This is an interdisciplinary approach which involves the collaboration of medical doctors, ethnobotanists, indigenous healers and communities [5,6,7]. The establishment of such a team permits the physician to interact with the healers in evaluation of the clinical diagnosis, while at the same time the ethnobotanist can identify the plants being used as medicines [7]. The information collected can then be compared with that in literature to assist in developing a list of priority plants for both phytochemical and pharmacological studies.

In this study, twenty plants used by traditional healers in Iringa region (Tanzania), were evaluated for cytotoxic activity on three human cancer cell-lines.

MATERIALS AND METHODS

Materials

RT-4 (human bladder carcinoma), HT29 (human colon adenocarcinoma) and A431 (human skin carcinoma) cell lines were obtained from American Type Culture Collection (Rockville, MD, USA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) was bought from Sigma (St. Louis, MO, USA), the cell culture media and ingredients and phosphate-buffered saline (PBS) were obtained from Gibco (Gibco BRL, Paisley, Scotland). Microtitre tissue culture plates were bought from Falcon (NJ, USA) and dimethylsulfoxide (DMSO) was obtained from Sigma (Poole, Dorset, England).

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Collection of plant materials

Plants used in this study identified by the voucher numbers are indicated in Table1. They were collected by Mr. E.B. Mhoro, and their respective voucher specimens were deposited in the Herbarium of the Institute of Traditional Medicine, Muhimbili University College of Health Sciences, Dar es Salaam, Tanzania.

Plant preparation

The plant materials were dried in open air under the sun, ground into powders and then 50 g extracted with 20% aqueous ethanol. The extracts were dried by rotary evaporation and the remaining traces of water were removed by freeze-drying. The dry extracts, ranging from 0.5 to 1.0 g/50 g of starting material were stored in plastic containers at –20 °C, until needed for testing.

Cell culture

Human bladder carcinoma (RT-4), human colon adenocarcinoma (HT29), and human skin carcinoma (A431) cells were grown at 37 °C in humidified 5% CO_2 and 95% air atmosphere in Minimum Essential Medium (MEM) with Earle's Salt containing 2 mM L-glutamine, 1% antibiotic/antimycotic solution, 1% non-essential amino acids, 1% anti-PPLO agent, and 10% fetal calf serum.

Antiproliferative assay

Extracts were first dissolved in DMSO to make stock solutions and then diluted in culture medium to yield an extract solution with a final DMSO concentration of 0.1% v/v. This concentration of DMSO did not affect cell viability. Cells were seeded onto 96-well microtitre tissue culture plates at 5 x 10^3 cells per well and incubated for 24 h at 37 °C. The medium was replaced with fresh medium containing different concentrations of extracts (100 µg/ml and 10 µg/ml) or the vehicle. The cells were then incubated at 37 °C for 72 h. Afterwards, the extract-containing medium was removed and cell proliferation was determined. Cell proliferation was determined by using the MTT dye reduction assay. MTT was dissolved in phosphate buffered saline (PBS, 0.01M; pH 7.4) and added to the cells (1 mg/ml) and the plates were incubated at 37 °C for 4 h. MTT was carefully removed and the resulting formazan crystals were dissolved in DMSO and added onto the wells (100 µl/well). The plates were placed on a shaker for 2 h and then read on a microtitre plate reader (SLT, Salzburg, Austria) at 550 nm. The results are expressed as percentage cell survival as compared to controls. All experiments were performed at least three times.

RESULTS

Table 1 shows the names of the twenty plants used, voucher numbers, the parts collected, and the ethnomedical claims for which they were collected. The ethnomedical uses included malaria (40%), epilepsy (40%), diabetes (30%), bilharzia (25%), hypertension (20%), HIV (15%), cancer (15%), and skin diseases (15%). Sixty percent (60%) of the plants had more than one ethnomedical use. Three of the plants collected, *Asparagus africanus*, *Cassia abbreviata* and *Ziziphus abbyssinica* were claimed to be used for the treatment of cancer. Table 2 shows the effect of the plant extracts on cell lines. At 100 µg/ml *Albizia harveyi*, *Albizia anthelmintica*, *Dalbergia nitidula*, *Euphorbia grantii* and *Rauvolfia caffra* exhibited up to 50% cytotoxicity on the three cell lines. Only one plant, *Albizia harveyi* showed a significant cytotoxic activity on the RT-4 cell line (percentage survival~23%) at 10 µg/ml. The other plant which performed well at 10 µg/ml is *Dalbergia nitidula*, with 39 and 34% cytotoxicity on RT-4 and HT-29 cells, respectively.

DISCUSSION

Three of the plants in this study, *Asparagus africanus*, *Cassia abbreviata* and *Ziziphus abbyssinica* are used by the local communities in Iringa for the treatment of cancer, but they did not show activity on the three human cancer cell lines. *Lannea stuhlmannii*, which has similar claims [8], also gave negative results like two earlier studies, one using brine shrimps [9] and another using HeLa (cervical carcinoma cell line) and A431 cell lines [10]. *Ximenia caffra* and *Cassia abbreviata* have also been retested but, it seems the two plants growing in Iringa were not as active as was expected from the previous study [10]. *Ximenia caffra* was inactive on the 3 cell lines at 10 and 100 µg/ml. The results for *Ximenia caffra* are

Table 1. The list of the plants, parts used and the diseases for which they were collected

Binomial name (Family)	Voucher no.	Vernacular name	Part collected	Disease for which collected
Acacia kirkii Oliv. (Mimosaceae)	IMPP 001-0031	Mbata/mgunga	Roots	Malaria, HIV, bilharzia, skin diseases
Albizia harveyi E. Fourn. (Mimosaceae)	MJ.152	Msisina/msisimizi	Roots	Skin diseases
Albizzia anthelmintica Brogn. (Mimosaceae)	MJ.98	Mfuleta	Stem bark	Diabetes
Asparagus africanus Lam. (Asparagaceae)	IMPP 001-0054	Mkalakanga	Roots	Hypertension, cancer, epilepsy
Cassia abbreviata Oliv. (Caesalpiniaceae)	IMPP 001-0010	Mmulimuli	Roots	Malaria, cancer, diabetes
Catunaregum spinosa (Thunb) Tirvengadum ssp. *taylori* (S. Moore) Verdc. (Rubiaceae)	IMPP 001-0022	Mpongolo	Roots	Epilepsy, skin diseases, HIV
Combretum molle G. Don (Combretaceae)	MJ.155	Mlama/mbadilo	Roots	Malaria, epilepsy
Dalbergia nitidula Kabll. (Fabaceae)	IMPP 001-0030	Mlengwe	Roots	Epilepsy
Euphorbia candelabrum Kotschy var. *bilocularis* (N.E.Br) S.Carter Euphorbiaceae)	IMPP 001-0047	Mnangali	Roots	Hypertension
Euphorbia grantii Oliv. (Euphorbiaceae)	IMPP 001-0057	Kidwenyi	Roots	Diabetes, bilharzia
Gardenia ternifolia Schumach & Thonn. ssp. *Jovis-tonantis* (Welw.)Verdc. (Rubiaceae)	IMPP 001-0028	Kilemandembwe	Roots	Epilepsy, hypertension
Lannea stuhlmannii Engl. (Anacardiaceae)	MJ.214	Mumbu	Stem	NIDDM
Momordica calantha Gilg. (Cucurbitaceae)	IMPP 001-0040	Mtundwa	Leaves	Epilepsy, bilharzia
Myrica salicifolia Hochst. (Myritacea)	IMPP 001-0007	Mkufwa	Roots	Bilharzia, malaria, diabetes
Piliostigma thornningii (Schumach.) Milne-Redh (Caesalpiniaceae)	MJ.159	Msegese	Stem	
Rauvolfia caffra Sond. (Apocynaceae)	IMPP 001-0023	Mvelevele	Roots	Malaria, epilepsy
Strychnos cocculoides Baker. (Loganiaceae)	IMPP 001-0001	Mtangadasi	Fruits	Malaria
Vepris glomerata (H. Hoffm.) Engl. var *glomerata* (Rutaceae)	IMPP 001-0012	Mtulisege	Roots	Malaria
Ximenia caffra Sond. (Olaceae)	MJ.113	Mdunula	Roots	Bilharzia`, hypertension, epilepsy
Ziziphus abyssinica A.Rich. (Rhamnaceae)	IMPP 001-003	Mtanula	Roots	Cancer, malaria, HIV, diabetes

Table 2: Cytotoxicity (MTT) assay of plant extracts (100 and 10 µg/ml) on human carcinoma cell lines (RT-4, HT-29 and A431). The results are expressed as percentage cell proliferation as compared to control cell lines. The results are an average of three independent experiments

20 % aqueous ethanol extract of	RT-4		HT-29		A431	
	100 µg/ml	10 µg/ml	100 µg/ml	10 µg/ml	100 µg/ml	10 µg/ml
Acacia kirkii	79	87	89	95	67	92
Albizia harveyi	21	23	58	69	75	87
Albizia anthelmintica	55	97	36	91	8	106
Asparagus africanus	100	88	57	86	63	83
Cassia abbreviata	100	88	67	110	92	96
Catunaregum spinosa	89	113	98	100	81	106
Combretum molle	79	81	67	100	51	74
Dalbergia nitidula	32	61	56	66	25	80
Euphorbia candelabrum	100	76	94	84	71	71
Euphorbia grantii	31	100	51	86	21	100
Gardenia ternifolia	100	94	96	100	98	102
Lannea stuhlmannii	100	98	99	98	92	88
Momordica calantha	100	100	32	100	47	90
Myrica salicifolia	75	100	98	100	65	97
Piliostigma thonningii	100	89	98	93	69	81
Rauvolfia caffra	32	72	11	90	17	83
Strychnos cocculoides	89	85	98	90	100	100
Vepris glomerata	73	85	66	93	71	102
Ximenia caffra	100	91	85	96	100	83
Ziziphus abyssinica	100	88	88	98	69	102

also in disagreement with a previous study which gave an LC_{50} of 0.7 µg/ml on brine shrimps [9]. *Gardenia ternifolia* [11,12], *Mormodica calantha* [13], *Rauvolfia caffra* [14], *Dalbergia nitidula* [15, 16], and *Euphorbia candelabrum* [13] are used for treatment of swellings, external tumors or for dressing wounds. At 10 µg/ml the only plant which inhibited cell proliferation by more than 50 % was *Albizia harveyi*, while *Euphorbia grantii*, *Dalbergia nitidula* and *Rauvolfia caffra* showed reasonable inhibition at 100 µg/ml.

CONCLUSION

The results show that 19 (95%) of the plant extracts tested are non toxic. One plant (5%), *Albizia harveyi* showed cytotoxic activity on one of the cell lines used, in agreement with the accepted detection level of biological activity by chance. Bioassay guided fractionation of the plant extracts to identify active compound(s) is suggested.

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