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Antiproliferative Effect of Plant Extracts used in Tanzanian Traditional Medicine

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Thirty plants were collected from traditional healers for cytotoxicity screening. Only six plant extracts were found to have cytotoxic activity *in vitro* on the three human cell lines, RT-4 (transitional cell papillary carcinoma, urinary bladder), HT29 (colon adenocarcinoma) and A431 (epidermoid carcinoma). There was no correlation between the use of plants in traditional medicine and the cytotoxic activity found. The reason could in part, be due to the inability of traditional healers to diagnose cancer. The one plant claimed to be used to treat cancer by the traditional healers was found to be inactive in the bioassay (>63% proliferation). Isolation of the active ingredients and testing on different cell lines is necessary in determining selective cytotoxicity.

Key words: Tanzanian medicinal plants, antiploriferative effect, cytotoxicity

INTRODUCTION

The cure for different types of cancers is believed to be found in the plant kingdom. Certain plant-derived compounds are known to be of value as anti-tumor agents. Investigators involved in the development of drugs from natural products argue that there is a close relationship between a traditional preparation and a drug obtained from the same plant. In one study [1], a correlation between the traditional uses of some plants with the pharmacological action of the isolated drug for 119 substances extracted from plant sources was analyzed. Of the 119 plant-derived drugs, 88 were discovered as a result of chemical studies to isolate the active substances responsible for the use of the original plants in traditional medicine. Traditional medicine is therefore a good approach for discovering other useful drugs from plants. Regrettably, it is not good for selecting plants in antitumor screening programs. The major disadvantage of this approach is the inability of traditional healers to diagnose cancer specifically because the types of cancers that are the big killers of man are tumors of the internal organs. It can however be of importance in the discovery of novel treatments for external tumors like those in oral cavity, skin cancers, warts and external growths which are easily detected [2].

In continuation with our efforts to screen Tanzanian traditional medicinal plants for cytotoxicity [3], an additional 30 plant extracts were evaluated for their cytotoxic potential *in vitro* on three human cell lines; RT-4 (Transitional cell papillary carcinoma, urinary bladder, human), HT29 (colon adenocarcinoma) and A431 (human epidermoid carcinoma). The plants were collected from Morogoro region in Tanzania where they are used in treating various diseases including AIDS related illnesses [4].

MATERIALS AND METHODS

Plant materials: All plant samples were collected from Morogoro region in Tanzania and their respective voucher specimens are deposited at the Department of Pharmacognosy, Faculty of Pharmacy of the Muhimbili University College of Health Sciences (Tanzania).

Extraction: For each plant sample, plant materials were dried at room temperature and ground. Dry powdery plant samples were exhaustively extracted with methanol by maceration. Dry methanolic extracts were obtained after removing the solvent by evaporation under reduced pressure. *Cell culture and culture medium.* RT-4 (transitional cell papillary carcinoma, urinary

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bladder, human), HT29 (colon adenocarcinoma, human) and A431 (human epidermoid carcinoma) cell lines were obtained from the American Type Culture Collection (TCC). 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 solution, 1 % non-essential amino acids and 1 % anti-PPLO agent and 10 % fetal calf serum. The medium, cell culture ingredients and phosphate-buffer saline (PBS) were obtained from Gibco BRL, (Paisley, Scotland).

Dilutions of stock solutions was made in culture medium to yield final extract concentrations of 10 and 100 ug/ml with 0.1 % dimethylsulphoxide (DMSO). This concentration of DMSO does not affect the cell viability.

Cytotoxicity assay. Cells were seeded onto 96-well tissue microtitre culture plates at 5 x 10^3 cell per well and incubated for 24 h at 37° C. The cells were then exposed to 10 or 100 µg/ml of the extracts or just medium (controls). The cells were then incubated at 37° C for 72 h. Afterwards the extracts containing medium were removed and replaced with fresh medium. Cell proliferation was determined by the use of 3-(4,5-dimethylthiazol-2-yl)-2,5-dephenyl-tetrazolium bromide (MTT) (Sigma, St. Louis, MO, U.S.A.) dye reduction assay. MTT was dissolved in 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 100 µl of DMSO. The plates were read on a microtitre plate reader (SLT, Salzburg, Australia) at 550 nm. Experiments were performed in triplicates and the results were expressed as cell proliferation, as % of control.

RESULTS AND DISCUSSION

Table 1 shows a list of plants used in this study and the antiproliferative assay results on three human carcinoma cell lines; RT-4, HT-29 and A431. The results are the average of three independent experiments with less than 10% standard deviation. It can be seen from the table that six plants i.e. *Parinari culateilifolia, Trichila emetica, Aspilia mossambiscensis, Mytenus senegalensis, Lantana camara* and *Ficus mucuso* show pronounced cytotoxicity in all cell lines. None of them however was reported to be used in traditional medicine for the treatment of cancer or related illnesses. The plants are used in traditional medicine for treating various infectious diseases [4]. However, a useful cytotoxic agent is the one which shows a high degree of selective toxicity towards a particular tissue. Such an agent is even more useful if its toxicity is not a broad one but directed towards tumor cells of the tissue while sparing normal cells of the same tissue. Of interest in this regard could be *Ficus sycomoros* whose extract shows some kind of selective cytotoxicity on HT-29 (colon adenocarcinoma) cell line (<50% cell proliferation in both concentrations). The plant is used in traditional medicine for treatment of venereal diseases and stimulation of milk production in women. Isolation of active constituent(s) from the plant may give valuable information regarding its activity.

In general, the extracts were more cytotoxic to A431 (skin carcinoma) cells than to the other two cell lines. On the other hand *Ehretia amoena* was the only plant claimed by traditional healers to be used in treatment of cancer. However, according to results in table1, it is one of the least active plants (>63% cell proliferation). There are two major problems that investigators face in deciding what plant to collect for screening when interviewing traditional healers. One is that preparations from traditional healers are often made from one major plant and other plants. Researchers normally collect the main plants and omit the *other plants* that are reported by the traditional healer as minor combinations. It has been frequently shown that one of these other plants is the truly active plant [5]. Secondly, utilization and formulations made by the healers are neglected in assessment of efficacy and/or toxicity of medicinal plants [6]. It is also important to remember that water is the only solvent used by traditional healers, therefore only water-soluble compounds are extracted. Meaningful investigation however requires the isolation of cytotoxic compound(s) from the active plant extracts and testing on different cancer cell lines for selective toxicity.

Table 1. Antiproliferative Effect of Plant Extracts used in Tanzanian Traditional Medicine n human carcinoma cells (RT-4, HT-29 and A431)

PLANT EXTRACT	Part	Cell proliferation (% of control)[a]					
		RT-4 (μg/ml)		HT-29 (μg/ml)		A431 (μg/ml)	
		100	10	100	10	100	10
Acalypha fruticosa Forsk (Euphorbiaceae)	R	+	+	+	-	+	-
Afzelia guinensis Welw. (Caesalpiniaceae)	SB	-	-	+	-	+	-
Aspilia mossambiscensis Willd. (Compositae)	L	+++	++	+++	+	+++	+
Byrocarpus boivianiamus (Baill) Schiell (Connaraceae)	R	-	-	-	-	+	-
Catunaregan spinoa Tirvengadum ssp taylorii Verd. (Rubiaceae)	R	-	-	-	-	-	-
Crassocephalum bojeri Robyns (Compositae)	L	+	-	++	-	++	-
Cussonia zimmermanii Harms (Arariaceae)	SB	-	-	++	-	++	-
Delonix regia Raf (Caesalpiniaceae)	R	-	-	-	-	+	-
Diospyrus squarrosa Klotzsch (Ebenaceae)	R	+	-	++	-	++	-
Ehretia amoena Klotzsch (Boraginaceae)	R	+	-	+	-	+	-
Euclea racemosa Marr ssp schimperi White (Ebenaceae)	R	-	-	-	-	+	-
Ficus mucuso Ficalho. (Moraceae)	R	-	+	++	+	+++	+
Ficus sycomorus L. (Moraceae)	R	-	-	+++	++	+++	-
Gardenia ternifolia Schumach x Thonn ssp jovis tonantis Verdc (Rubiaceae)	R	-	-	-	-	-	-
Grewia hexamita Harv. (Tiliaceae)	R	-	-	-	-	-	-
Grewia microcarpa K. Schum (Tiliaceae)	ST	-	-	+	-	+	-
Grewia microcarpa K. Schum (Tiliaceae)	R	+	-	-	-	-	-
Lantana camara L. (Verbenaceae)	R	+	+	+++	+	+++	+
Monanthotaxis trichocarpa Verd (Annonaceae)	L/S	-	-	-	-	+	-
Mytenus senegalensis Exell (Celastraceae)	R	+	+	+	+++	++	+++
Ozoroa insignis Del. (Anacardiaceae)	R	+	-	-	-	+	-
Parinari culateilifolia Planch ex Benth. (Rosaceae)	R/B	++	++	+++	++	+++	++
Steganotaemia araliaceae Hochst. (Urticaceae)	SB	++	+	+	+	++	-
Strychnos innocua Del. ssp innocua var pubescens Solerel (Loganiaceae)	R	+	-	-	-	-	-
Syzygium cuminii Shells (Myrtaceae)	SB	-	-	-	-	-	-
Terminaria sericea DC. (Combretaceae)	R	++	-	++	-	++	-
Trichila emetica Vahl (Meliaceae)	B	+++	-	+++	+++	+++	++
Tylosema fassoglensis Torre & Hillc (Papilionaceae)	R	-	-	-	-	+	-
Xeroderris stuhlmannii Medonca & Souza (Papilionaceae)	SB	-	-	-	-	+	-
Ximenia americana L. var caffra Engl. (Olacaceae)	B	-	-	-	-	-	-

[a] -, indicates 100-75%; +, 75-50%; ++, 50-25%; and +++, 25-0% cell proliferation vs. control. The results are the average of three independent experiments with less than 10% standard deviations.
R = root, L = leaves, B = barks, ST = stem and SB = stem bark.

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