Anticancer Activity of Extracts from some Endemic Tanzanian Plants

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Plants have shown to be good sources of a variety of drugs for human ailments including cancer. Tanzania is rich in plant species most of which have not been investigated for any biological activity. In the continuing effort to screen Tanzanian plants for anticancer activity, plants were collected from Lindi region and extracts tested for the activity using two cell lines namely RT112 (Human bladder transitional cell carcinoma) and HeLa (Human cervical carcinoma). Of the 52 extracts from 26 plants of different families tested, 5 demonstrated potential activity on the cells. Extract X13 had an exceptionally high activity on both cell lines while extract X29 was highly active on HeLa cells. Fractionation and isolation of constituents from the extracts that have shown anticancer activity in these cell lines is recommended.

Key words: Medicinal plants, extracts, anticancer activity, screening

INTRODUCTION

Cancer is a cellular malignancy whose unique characteristic is loss of normal controls resulting in unregulated tissue growth, lack of differentiation, and ability to invade local tissues and metastasis [1]. Cancerous cells mass together to form a growth or proliferate individually throughout the body. They serve no function and may interfere with the function of the organ and sometimes invade neighbouring tissues. During metastasis, cancer cells enter the blood stream and are carried to distant parts of the body where they form other similar growths.

Attention is now being directed by pharmaceutical companies and environmentalists to the study and conservation of plants as a source of medicinally active agents. Clinically useful cancer chemotherapeutic agents derived from plants are well known. Few would doubt the major impact of vincristine on the field of cancer chemotherapy as the drug of choice for induction of remissions in acute childhood leukemias. Other plant derived anticancer agents are vinblastine also extracted from *Catharanthus roseus* as well as the semi-synthetic derivatives related to these, vindesine and vinzolidine. In addition is podophyllotoxin from *Podophyllum peltatum* and its semi-synthetic derivatives etoposide and teniposide that have proved to be effective chemotherapeutic agents [2].

Prospects of finding new anticancer drugs from plants is encouraging as shown by recent isolation of anticancer agents like taxol (paclitaxel) from the barks of *Taxus brevifolia* and its semi-synthetic analogue taxotere (docetaxel) that have been introduced as drugs for the treatment of ovarian and breast cancers respectively [3]. The ultimate effectiveness of any anticancer drug requires that it kills malignant tumor cells *in vivo* at doses that allow enough cells in the patient's critical tissue to survive so that recovery can occur. None of the anticancer drugs in current use have been able to achieve this effectively especially given that cancer chemotherapeutic agents are the most toxic drugs in clinical use.

Tanzania is rich in flora that has not been completely investigated for its potential as a source of effective anticancer drugs. In the effort to search for new anticancer agents from plants endemic to Tanzania, plant extracts from

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different parts of Tanzania were tested for cytotoxicity using the Brine shrimp lethality test and cancer cell lines [4-7]. A number of the extracts showed promising activity when tested on different cancer cell lines including breast, lung, melanoma, sarcoma and skin carcinomas [8-11]. Responsiveness of these cell lines are characterized by treatment with the plant extracts for cytotoxicity. The aim of this approach is to obtain cytotoxic agents that may demonstrate tumor specificity. In this paper the screening results for 52 plant extracts on HeLa (human cervical carcinoma) and RT112 (human bladder transitional cell carcinoma) cells are presented.

EXPERIMENTAL

Plant material: The plants for study were randomly collected from Lindi region tropical forest in southern Tanzania. Voucher specimens are deposited in the Herbarium of the Department of Botany, University of Dar-es-Salaam, Tanzania.

Cell culture: HeLa cells were obtained from American Type Culture Collection (Rockville, MD, USA) while RT112 cells were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany).

Reagents: The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma (St Louis, MO, USA). Minimum Essential Medium (MEM), L-glutamine, non-essential amino acids, penicillin, streptomycin, tylocin, amphotericin B, fetal calf serum (FCS) and phosphate-buffered saline (PBS) were obtained from Gibco BRL (Paisley, Scotland, UK). Microtitre tissue culture plates were purchased from Falcon (New Jersey, USA) and dimethylsulfoxide (DMSO) from Sigma (Poole, Dorset, England).

Lindi forest was chosen because being a rain forest, there are many endemic plant species giving a good chance of collecting new plants that have not been previously studied for anticancer activity. Care was taken not to recollect plants we had already screened for the activity in previous studies. This was possible with assistance of a botanist. Depending on the size of the plant, at least three samples (1 kg each) from each plant (Table 1) were taken. The plant parts collected were roots, stems or barks and leaves. After collection, plant samples were dried and ground to coarse powder.

Extraction: An amount of 50 g powdered plant material was taken from each plant part collected and dissolved in sufficient amount of methanol in a conical flask. The mixture was kept in contact at room temperature for five days shaking the flask frequently and then filtered. The solvent was left to evaporate in the air at room temperature yielding a methanol extract which was used for the anticancer screening experiments. A total of 52 extracts (X1–X52) were prepared from the different plant parts.

Cytotoxicity assay: The extracts were first dissolved in dimethyl sulphoxide (DMSO) to make stock solutions and then diluted in culture medium to yield extract solution with a final DMSO concentration of 0.1%. This final concentration of DMSO had been shown not to affect cell viability in preliminary experiments. 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 in humidified 5% CO_2 and 95% air atmosphere. The medium was then replaced with fresh medium containing different concentrations of extracts or the vehicle. The cells were then incubated at 37 °C for 72 h. The extract-containing medium was then removed and cell proliferation determined using the MTT dye reduction assay. MTT (1 mg/ml) was dissolved in PBS (0.01M, pH 7.4) and added to the cells and the plates incubated at 37 °C for 4 h. The MTT solution was then carefully removed and 100 µl of DMSO added to each well in order to dissolve the formed formazan crystals. The plates were read on a microtitre plate reader (SLT, Salzburg, Austria) at 550 nm. The results were expressed as a percentage of cell survival as compared to the control. All experiments were performed in triplicate.

Table 1: Plants collected from Lindi region and part(s) collected of each plant

Collection No.	Botanical name	Family	Plant part collected	Extract No.
1	*Pterocarpus tinctorius* Welw. Race *Megalocarpus*	Papilionaceae	Stem wood, roots, stem bark.	X7,38,42
2	*Dalbergia arbutifolia* Bak. ssp *arbutifolia*	Papilionaceae	Stem bark, roots	X5,36,51
3	*Manilkara discolor* (Sond.) J.H. Hemsl.	Sapotaceae	Stem bark, roots	X18,34
4	*Paranecepsia alchorneifolia* A.R.Sm	Euphorbiaceae	Stem bark	X6
5	*Cordia africana* Lam	Boraginaceae	Stem wood, leaves	X3,45,
6	*Guibourtia schliebenii* J. Leon	Caesalpiniaceae	Roots, leaves	X24,29
7	*Bauhinia macrocalyx* Harms	Caesalpiniaceae	Stem	X9
8	*Hugonia busseana* Engl.	Linaceae	Stem, roots	X22,47
9	*Cladostemon kirkii* Pax & Gilg.	Capparidaceae	Stem bark	X16,
10	*Monodora grandidieri* Baill.	Annonaceae	Stem wood, leaves	X20,37
11	*Clerodendrum sansibarense* Guerke ssp. *sansibarence*	Verbenaceae	Leaves, roots, leaves	X1,49
12	*Sterculia quinqueloba* (Garke) K. Schum.	Sterculiaceae	Stem bark, stems	X4,23
13	*Albizia amara* ssp *amara*	Mimosoideae	Roots, leaves, stem bark	X13,44,48
14	*Bombax rhodognaphalon* K. Schum.	Bombacaceae	Stem bark	X31
15	*Caloncoba welwitschii* Gilg.	Flacourtiaceae	Stem wood, root, leaves	X2,30,33
16	*Artabotrys brachypetalus* Benth.	Annonaceae	leaves	X21
17	*Drypetes natalensis* Hutch	Euphorbiaceae	Leaves, roots, stem	X8,46,52
18	*Acalypha ciliate* Forsk.	Euphorbiaceae	Roots, leaves, stem	X11,25,41
19	*Paramacrolobium caeruleum* J. Leon	Caesalpiniaceae	Stem, Stem bark	X10,50
20	*Mallotus oppositifolius* Muell.Arg. forma *oppositifolius*	Euphorbiaceae	Roots, stem bark	X12,27,39
21	*Paropsia grewioides* Mast.	Gentianaceae	Leaves, root, stem	X14,40,43
22	*Olax pentandra* Sleumer	Olacaceae	Leaves, stem	X26
23	*Dalbergia obovata* E.Mey	Papilionaceae	Leaves, stem	X19,35
24	*Diospyros usambarensis* F. White ssp.*usambarensis*	Ebenaceae	Stem wood	X28
25	*Mammea americana* L.	Cluciaceae	Root bark	X15
26	*Tetracela boiviniana* Baill.	Dilleniaceae	Roots, leaves	X17,32

Initially all the 52 extracts were tested for cell proliferation on RT112 cells at a concentration of 100 µg/ml extract. The resulting 14 most active plant extracts were then tested on RT112 and HeLa cells at a concentration of 10 µg/ml extract and activity compared to the control. From the results obtained, the four most active fractions were further tested at the lower concentrations of 5, 2, 1 and 0.1 µg/ml extract.

RESULTS AND DISCUSSION

Figure 1 shows the activity profile when survival fraction (% of control) is compared among the 8 initially active plant extracts on RT112 at 10 µg/ml concentration. Extracts X13, X15, X44 and X47 were the most active showing survival fraction of less than 50%. Fraction X13 was especially active with the survival fraction below 5%.

Figure 2 shows activity profile of 10 most active extracts tested on HeLa cells. In this case, five extracts displayed the highest activity with survival fraction being less than 30%. Extract X13 maintained good activity followed by extracts X44, X47, X15 and X29.

Figures 3 and 4 show the activity profile of four extracts (X13, 15, 44 and 47) tested on both cell lines at a concentration of 100, 10, 5, 2, 1 and 0.1 µg/ml. The survival fraction is plotted against the logarithm to base 10 of concentrations.

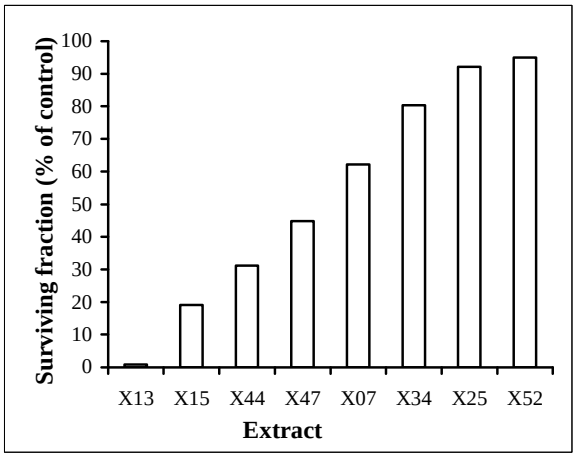

Figure 1: Cytotoxicity of the plant extracts (10 µg/ml) on RT112 cells.

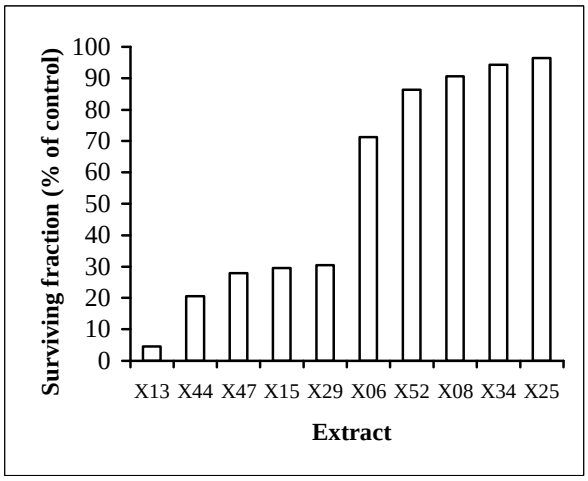

Figure 2: Cytotoxicity of extracts (10 µg/ml) on HeLa cells.

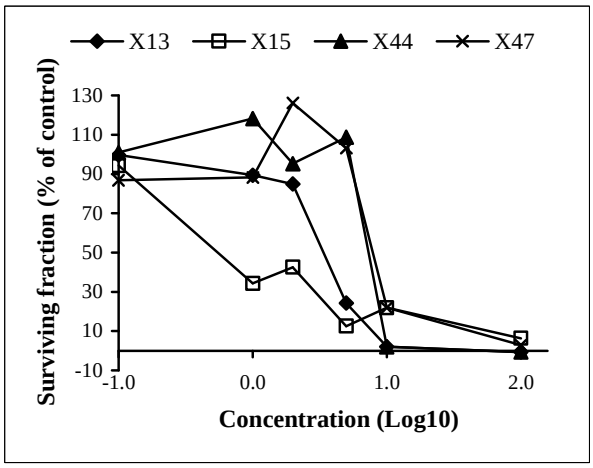

Figure 3: Cytotoxicity of the most active extracts (100-0.1 µg/ml) on RT112 cells.

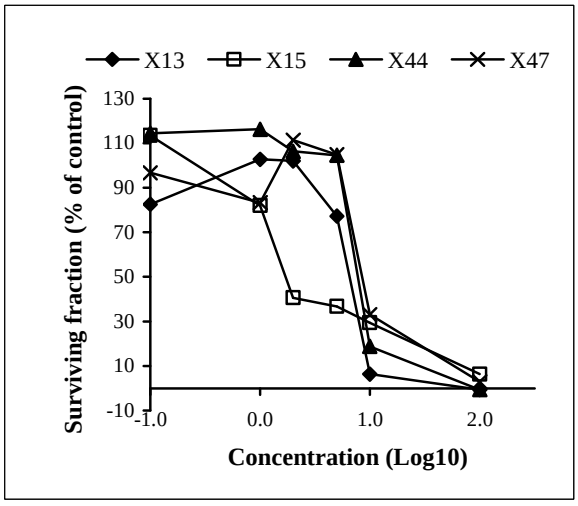

Figure 4: Cytotoxicity of the most active extracts (100-0.1µg/ml) on HeLa cells.

The 52 extracts prepared for study were screened on HeLa and RT112 cell lines. It can be seen that 6 (11.5%) have shown potential activity on the cancer cell lines tested. Out of the 6 extracts, X13 was the most active on RT112 followed by X15, X44 and X47 in that order. Extract X13 was also the most active on HeLa cells followed by X44, X47, X15 and X29. Notably, extract X29 was active only on HeLa cells but inactive on RT112 cells. This is an important observation given that an effective anticancer drug should demonstrate tumor specificity. An extract that demonstrates cytotoxicity on all cell lines might not be a potential lead for an anticancer drug because it lacks selective cytotoxicity. It may kill the non-cancerous cells as well. On the same note, extract X15 was less active on HeLa cells compared to the other fractions but selectively active on RT112.

CONCLUSION

Although the cytotoxicity demonstrated by the 6 extracts is potentially useful, further tests on other cancer cell lines need to be done to determine their tumor specificity. Fractionation of the extracts should be done so as to isolate the active ingredients. Subsequently, the active compounds can further be subjected to structure modifications to reduce toxicity and/or enhance activity.

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