High throughput screening of South African plants for anti-cancer properties

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INTRODUCTION

Plants have a long history of use in the treatment of cancer and over 60% of currently used anti-cancer agents are derived in one way or another from natural sources[1]. South Africa has a rich plant biodiversity with only a limited number reported for the treatment of cancer[2]. As a result, a collaborative research programme was initiated between the CSIR and the National Cancer Institute (NCI) in the USA, aimed at the screening of plant extracts and identification of potentially new anti-cancer drug leads. To date, 7 500 randomly selected plant extracts representing 700 taxa were tested against a panel of three human cancer cell lines (breast MCF7, renal TK10 and melanoma UACC62) at the CSIR. Plant extracts that exhibited anti-cancer activity against these three human cell lines were then screened by the NCI against 60 human cancer cell lines organised into sub-panels representing leukaemia, non-small cell lung, colon, central nervous system, melanoma, ovarian, renal, prostate and breast cancer lines.

We have previously reported on South African plants having moderate anti-cancer activity[3]. We now wish to report on those plants that have shown potent anti-cancer activity arising from our ongoing high throughput anti-cancer screening programme at the CSIR.

MATERIALS AND METHODS

Plant material

Plant material collections were conducted from various regions in South Africa and voucher specimens deposited and identified at the South African Biodiversity Institute (SANBI). An average of three plant part samples were collected from the same terrestrial plant specimen and each part constituted a separate physical sample.

Extraction methods

Plant samples were dried in an oven at 30-60 °C. Dried material was ground to a coarse powder using a hammer mill and (100-500g) was sequentially extracted with dichloromethane (DCM), DCM/methanol (MeOH), MeOH and purified water. Organic extracts were concentrated by rotary vacuum evaporation and then further dried *in vacuo*. The aqueous extracts were concentrated by freeze-drying. All extracts were stored at -20 °C.

In vitro anti-cancer screening (CSIR and NCI)

The high throughput method adopted at the CSIR against 3-cell line panels allows the screening of 280-380 samples at one dose (100 ppm) or 60-70 samples at five doses over a week using the protocol of the Drug Evaluation Branch, National Cancer Institute[4,5,6]. End point determinations were made with a protein-binding dye, Sulforhodamine B (SRB) (see **Figure 2**). Extracts, which reduced the growth of two of the cell lines by 75% or more, were further tested at five concentrations ranging from 6.25-100 ppm with Etoposide used as a positive standard. The results of the five dose assays were reported as TGI (total growth inhibition) and extracts that exhibited TGI < 6.25 ppm were considered to be potent (see **Figure 1**). Extracts falling in this category were subjected to further *in vitro* testing for selective cytotoxicity against panels of 60 human cancer cell lines at the NCI. Results from NCI were reported as mean log10 functions of the three response parameters, GI_{50} (50% growth inhibition), TGI (drug concentration that is indicative of the cytostatic effect of the test agent), and LC_{50} (50% lethal concentration indicative of the cytotoxic effect of the test agent), calculated for each cell line.

RESULTS AND DISCUSSION

Thirty-two plant extracts were found to demonstrate potent anti-cancer activity, representing 24 different plant taxa, which is a hit rate of 3,4% based on the number of taxa screened (**Table 1**). Among the 32 potent extracts, six belong to the phylum Apocynaceae, representing three plant species. The plant species *Acokanthera oppositifolia* and *Gomphocarpus fruticosus* are reported in literature as sources of toxic cardiac glycosides, which cause livestock poisoning in South Africa[7].

The phylum Crassulaceae, *Kalanchoe paniculata* and *Cotyledon orbiculata spp. oblonga*) is reported to contain bufadienolides and these are toxic to livestock and cause the well-known krimpsiekte[8]. The Solanaceae family, representing three *Solanum species* (0,4% hit rate), is a source of steroidal alkaloids and bioassay-guided fractionation of the plant extract of *Solanum aculeatissimum* yielded Solasonine with reported cytotoxicity and cancer-related activity[8]. The highest hit rate in this study was from the phylum Asteraceae, which is rich in sesquiterpene lactones and representing four plant species (0,6%). Ursolic acid was isolated from *Cussonia paniculata*. Triterpenoid acids such as oleonolic and ursolic acid are common plant constituents and associated with anti-tumor activities[1]. A cytotoxic *ent*-kaurene diterpenoid, 13-methoxy-15-oxozoapatlin, was isolated from the bioassay-guided fractionation of *Parinari curatellifolia*. The structure and cytotoxicity was published by Kinghorn[9] and the compound showed selectivity for leukaemia cell lines. Plumbagin was isolated from the organic extract of *Plumbago zeylanica* (Plumbaginaceae) and *in vitro* cytotoxicity against melanoma and breast cancer cell lines was demonstrated by Nguyen[10].

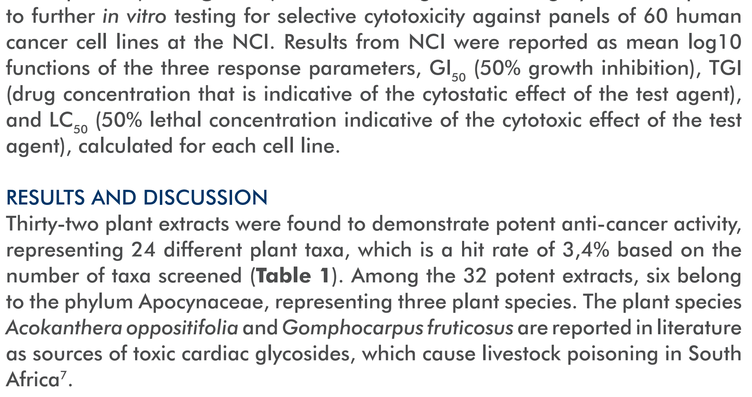


Figure 1: Graph of a plant extract showing potent anti-cancer activity

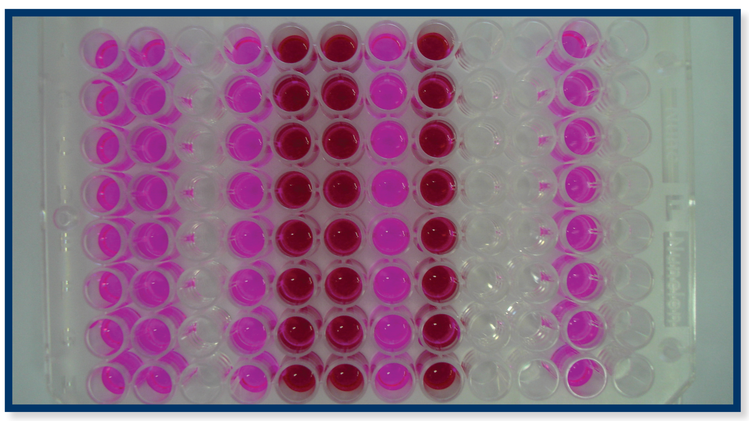


Figure 2: Cancer cells in the 96-well microlitre plates

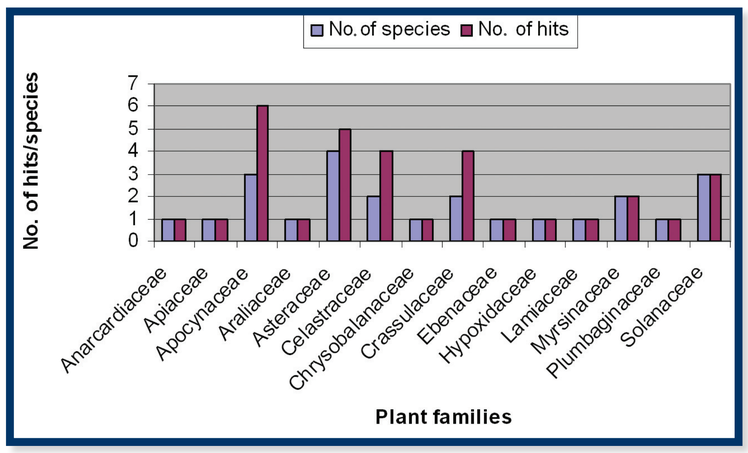


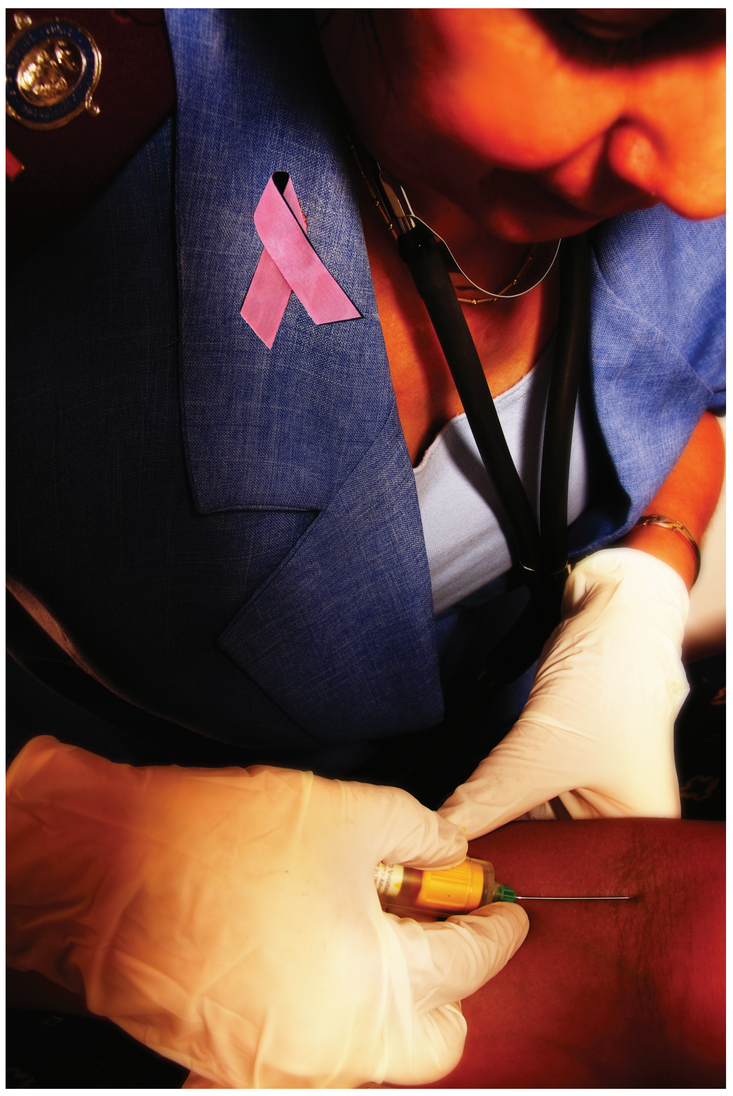
Figure 3: Graph representing the potent hit rate of plant specimens from different plant families

Table 1: Plant extracts exhibiting potent *in vitro* anti-cancer activity at the CSIR

Family	Plant species	CSIR sample number	Plant part	Extraction solvent	NCI result
ANACARDIACEAE	*Rhus lancea*	P00950A	whole plants	DCM	Not tested
APIACEAE	*Steganotaenia araliacea ssp. araliacea*	P00746B	leaves	DCM:MeOH	Not tested
APOCYNACEAE	*Gomphocarpus fruticosus*	P00552A	fruits	DCM	Not tested
APOCYNACEAE	*Acokanthera oppositifolia*	P00651B	fruits	DCM:MeOH	Not tested
APOCYNACEAE	*Acokanthera oppositifolia*	P00653A	stems	DCM	Not tested
APOCYNACEAE	*Acokanthera oppositifolia*	P00654B	roots	DCM:MeOH	Potent
APOCYNACEAE	*Gomphocarpus fruticosus*	P00786A	leaves, stems	DCM	Not tested
APOCYNACEAE	*Gomphocarpus physocarpus*	P00463B	roots	DCM:MeOH	Moderate
ARALIACEAE	*Cussonia paniculata*	P00656A	leaves	DCM	Moderate
ASTERACEAE	*Zinnia peruviana*	P00320A	whole plants	DCM	Potent
ASTERACEAE	*Tithonia diversifolia*	P00633A	leaves	DCM	Not tested
ASTERACEAE	*Tithonia diversifolia*	P00635B	stems	DCM:MeOH	Not tested
ASTERACEAE	*Athrixia elata*	P00204A	leaves, seeds	DCM	Moderate
ASTERACEAE	*Xanthium strumarium*	P00483B	stems	DCM:MeOH	Moderate
CELASTRACEAE	*Gymnosporia tenuispina*	P00316B	whole plants	DCM:MeOH	Potent
CELASTRACEAE	*Gymnosporia tenuispina*	P00317B	leaves, flowers	DCM:MeOH	Potent
CELASTRACEAE	*Catha edulis*	P00469A	roots	DCM	Moderate
CELASTRACEAE	*Catha edulis*	P00470A	leaves	DCM	Potent
	Parinari curatellifolia	P00256A	roots	DCM	Moderate
CRASSULACEAE	*Kalanchoe paniculata*	P01052B	roots	DCM:MeOH	Not tested
CRASSULACEAE	*Kalanchoe paniculata*	P01056B	leaves	DCM:MeOH	Not tested
CRASSULACEAE	*Cotyledon orbiculata spp. oblonga*	P02645B	stems	DCM:MeOH	Not tested
CRASSULACEAE	*Cotyledon orbiculata spp. oblonga*	P02650B	roots	DCM:MeOH	Moderate
EBENACEAE	*Diospyros whyteana*	P00283A	roots	DCM	Weak
HYPOXIDACEAE	*Hypoxis rigidula spp. pilosissima*	P00282A	stems	DCM	Weak
LAMIACEAE	*Plectranthus verticillatus*	P01978A	whole plants	DCM	Not tested
MYRSINACEAE	*Rapanea melanophloeos*	P00234A	not noted	DCM	Moderate
MYRSINACEAE	*Myrsine africana*	P00965A	roots	DCM	Moderate
PLUMBAGINACEAE	*Plumbago zeylanica*	P00631B	leaves	DCM:MeOH	Moderate
SOLANACEAE	*Solanum aculeatissimum*	P00095B	leaves	DCM:MeOH	Moderate
SOLANACEAE	*Solanum panduriforme*	P00893C	stems	H_2O	Not tested
SOLANACEAE	*Solanum tomentosum*	P01294B	stems	DCM:MeOH	Not tested

Extraction solvent: DCM: Dichloromethane, MeOH: Methanol, H_2O: Water
CSIR's criteria: Potent: TGI< 6.25 µg/mL for 2 to 3 cell lines
NCI's criteria: Weak: log GI_{50} > 1.10 to 1.5
Moderate: log GI_{50} > 0 to 1.10
Potent: log GI_{50} < 0.

Over 60% of currently used anti-cancer agents are derived from natural resources. A CSIR study identified 32 plant extracts from 7500 randomly-selected plants exhibiting potent activity against three cancers.



CONCLUSION

Among the 32 plant extracts exhibiting potent activity in this study, the highest hit rate was observed for the family Asteraceae (see **Figure 3**), that is a known source of triterpenoids and sesquiterpene lactones. Results from this study led to the identification of known metabolites indicated by literature searches and were either patented or published for their use as anti-cancer agents. Perhaps the most notable observation from the results is that although the extracts of these taxa were randomly selected during the screening programme, 88% of these are reported to be used medicinally.

ENDNOTE

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