INVESTIGATION OF SOUTH AFRICAN PLANTS FOR ANTICANCER PROPERTIES

G. Fouche*, E. Khorombi, N. Kolesnikova, V.J. Maharaj,
R. Nthambeleni and M. van der Merwe

Bioprospecting, Biosciences, CSIR, PO Box 395, Pretoria, 0001, RSA.
* Corresponding Author: gfouche@csir.co.za

Summary

A collaborative research programme between the Council for Scientific and Industrial Research (CSIR) in South Africa and the National Cancer Institute (NCI) in the USA aimed at the screening of plant extracts and identification of potentially new anticancer drug leads was initiated in 1999. Plant extracts that exhibited anticancer activity against a panel of three human cell lines (breast MCF7, renal TK10 and melanoma UACC62) at the CSIR were screened by the NCI against sixty human cancer cell lines organized into sub panels representing leukaemia, melanoma and cancer of the lung, colon, kidney, ovary and central nervous system.

To date 7500 randomly selected plant extracts representing 700 taxa were screened for anticancer activity at a single concentration of 100 µg/mL. Extracts which exhibited a growth inhibition of above 75% for two or more of the cell lines (GI_{75}) were advanced into the dose response assay at concentrations ranging from 6.25-100 µg/mL. Extracts which exhibited a total growth inhibition (TGI) of less than 6.25 µg/mL for at least two cell lines where regarded as potent. The results indicated that a hit rate of 3.4% was obtained based on the number of taxa screened. Although the extracts of these taxa were randomly selected during the screening programme, 88% of these are reported to be used medicinally. The potent plant extracts were evaluated for efficacy at the NCI against a panel of sixty human cancer cell lines over a defined range of concentrations to determine the relative degree of growth inhibition against each cell line.

Desktop literature investigations aimed at establishing information on the scientific validation of the plants demonstrating potent anticancer activity were conducted. The extracts of taxa with limited scientifically published information for their anticancer properties were subjected to bioassay-guided fractionation and the active constituents were isolated

and identified. Results from this study led to the isolation and identification of known metabolites that have been described in the literature studies and were either patented or published for their use as anticancer agents. Anticancer activity was demonstrated for several metabolites and a few examples are discussed. These anticancer screening results mirrored the NCI experience where essentially, no new plant-derived clinical anticancer agents had been found from plants since the discovery of the taxanes and camptothecins in the early (1960-1980) program (1). Based on the outcome of this screening programme, a strategy was employed to target endemic plant species as well as plant species containing selected classes of compounds. An evaluation of these plant species is ongoing.

Key words**:** Plants, Cancer, South Africa

Introduction

South Africa which comprises of less than 1% of the world's land surface contains 8% of its plant species. This rich plant biodiversity, with over 20 000 different species, is a great source of interest to the scientific community (2). Plants have a long history of use in the treatment of cancer. It is significant that over 60% of currently used anticancer agents are derived in one way or another from natural sources (1).

The potential of using natural products as anticancer agents was recognized in the 1950s by the U.S. National Cancer Institute (NCI) and has since made major contributions to the discovery of new naturally occurring anticancer agents. In spite of the success of natural-products approach to anticancer drug discovery, reports on plants used for the treatment of cancer are rare in South Africa (3). As a result, a collaborative research programme was initiated between the Council for Scientific and Industrial Research (CSIR) in South Africa and the NCI, aimed at the screening of plant extracts and identification of potentially new anticancer drug leads.

An anticancer screening technology was implemented at the CSIR in 1999 with a panel of three human cancer cell lines namely, breast MCF7, renal TK10 and melanoma UACC62. These cell lines were selected because of their high sensitivity to detect anticancer activity. Plant extracts that exhibited anticancer activity against these three human cell lines were then screened by the NCI against sixty human cancer cell lines organized into sub panels representing leukaemia, melanoma and cancer of the lung, colon, kidney, ovary and central nervous system.

Materials and Methods

Plant material

Plant collections were conducted from various regions in South Africa by qualified plant collectors. The collection contractor(s) provided an average of 3 plant part samples (e.g. leaves and stems, roots) from the same terrestrial plant specimen collected and each part constituted a separate physical sample. Voucher specimens were deposited and identified at the South African National Biodiversity Institute (SANBI).

Extraction methods

Plant samples were dried in an oven at 30-60 ºC and the drying time and temperature varied depending on the nature of the plant part. Dried material was ground to a coarse powder using a hammer mill and stored at ambient temperature prior to extraction. Powdered plant material (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 at ambient temperature for 24 hours. The aqueous extracts were concentrated by freeze-drying. All extracts were stored at -20 ºC.

In vitro anticancer screening (CSIR and NCI)

The extracts and compounds were assayed in the 3-cell line panel consisting of TK10 (renal), MCF7 (breast), and UACC62 (melanoma). The primary anticancer assay was performed at the CSIR in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute (4,5,6). The extracts or compounds were tested at a single concentration (100 ppm) and the culture was incubated for 48 h. End point determinations were made with a protein-binding dye, Sulforhodamine B (SRB). The growth percentage was evaluated spectrophotometrically versus controls not treated with test agents. Results for each extract were reported as the growth percentage of the treated cells, compared to that of the untreated control cells. All the extracts which reduced the growth of two of the cell lines by 75% or more, were further tested at 1/2 log serial dilutions of five concentrations ranging from 6.25-100 ppm. The results of five dose screening were reported as TGI (total growth inhibition). The biological activities were separated into 4 categories: inactive (TGI >50 ppm), weak activity (15 ppm< TGI <50 ppm), moderate activity (6.25 ppm< TGI <15 ppm) and potent activity (TGI <6.25 ppm).

Extracts which demonstrated moderate and potent activity were selected for further *in vitro* testing for selective cytotoxicity against panels of human cancer cell lines at the NCI. The panel of 60 cell lines included leukemia (L) lines, non-small cell lung cancer (NSCLC) lines, colon cancer (CL) lines, central nervous system cancer (CNSC) lines, melanoma (M) lines, ovarian cancer (OC) lines, renal cancer (RC) lines, prostate cancer (PC) lines and breast cancer (BC) lines. The results from NCI were reported as mean $\log_{10}$ 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

Between late 1998 and 2005, the Bioprospecting research group of the CSIR coordinated the collection of approximately 22 000 samples of higher plants (which included multiple parts per single plant specimen) which were processed and extracted. An average of 3 plant part samples were collected for each plant specimen and an average of 3 extracts (aqueous and two organic solvents) were prepared from each sample. To date 7500 randomly selected plant extracts representing 700 taxa were tested in the three cell line pre-screen, which was performed at a nominal single dose of 100 µg/mL. Based on the literature investigation of the taxa, only selected species were tested at the NCI. Those taxa and their corresponding extracts which were identified as having potent anticancer activity are shown in Table 1.

Table 1: Plant extracts exhibiting potent i*n vitro* anticancer 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
CHRYSOBALANACEAE	*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
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.

Discussion

The 32 plant extracts which demonstrated potent anticancer activity, represent 24 different plant taxa which is a hit rate of 3.4% based on the number of taxa screened. Desktop literature investigations aimed at establishing information on the scientific validation of the plants as anticancer treatments identified 11 taxa with limited published information for their anticancer properties. Among the 32 potent extracts, 6 belong to the phylum Apocynaceae, representing 2 plant specimens. These plant species, *Acokanthera oppositifolia* and *Gomphocarpus fruticosus* are sources of toxic cardiac glycosides, which are the most important of all causes of livestock poisoning in South Africa (7). These plant species were not fractionated and researched. This represents 0.3% of the total number of plant species screened. Interestingly, high hit rates were also observed for the phylum, Apocynaceae by the NCI, but this was attributed to other genera tested containing indole alkaloids for example *Cathanthus roseus* (8). The phylum Crassulaceae, also represented in this list (0.3% of the total plant species screened, 2 plant species, *Kalanchoe paniculata* and *Cotyledon orbiculata spp. oblonga*) is reported to contain bufadienolides and these are toxic to livestock and cause the well-known krimpsiekte (7). Bufadienolides are cardiotoxic and these two plant species were not further researched. The Solaneceae family, representing 3 *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 4 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).

Perhaps the most notable observation from the results is that although the taxa and their extracts selected were randomly chosen, 88% of these taxa are reported to be used medicinally. Cancer, as a specific disease entity, is likely to be poorly defined in terms of folklore and traditional medicine. This is in contrast to other plant-based therapies used in traditional medicine for the treatment of afflictions such as malaria and pain, which are more easily defined.

Results from this study led to the identification of known metabolites indicated by literature studies and were either patented or published for their use as anticancer

agents. These anticancer screening results mirrored the NCI experience where essentially, no new plant-derived clinical anticancer agents had been found from plants since the discovery of the taxanes and camptothecins in the early (1960-1980) program (1). Based on the outcome of this screening programme, a strategy was employed to target endemic plant species as well as plant species containing selected classes of compounds. An evaluation of these plant species is ongoing.

Acknowledgements

The authors would like to thank the South African National Biodiversity Institute (SANBI) for the identification of plant specimens and the Council of Scientific and Industrial Research (CSIR) for financial grants.

References

1. Cragg GM, Newman DJ. Plants as a source of anticancer agents. *J Ethnopharmacol* 2005; 100: 72-79.
2. Cherry M. South Africa—Serious about Biodiversity Science. *Public Library of Science Biol.* 2005; 3: e145 DOI: 10.1371/journal.pbio.0030145.
3. Steenkamp V, Gouws MC. Cytotoxicity of six South African medicinal plant extracts used in the treatment of cancer, *South African Journal of Botany.* Article in press, 2006.
4. Monks A, Scudiero D, Skehan P, et al. Feasibility of a High-Flux Anticancer Drug Screen using a Diverse Panel of Cultured Human Tumor Cell Lines. *Journal of the National Cancer Institute* 1991; 83: 757–766.
5. Kuo SC, Lee HZ., Juang JP, et al. Synthesis and cytotoxicity of 1,6,7,8-substituted 2-(4'-substituted phenyl)-4-quinolones and related compounds: Identification as antimitotic agents interacting with tubulin. *J Med Chem* 1993; 36: 1146–1156.
6. Leteurtre F, Kohlhagen G, Paull KD, Pommier Y. Topoisomerase II inhibition and cytotoxicity of the anthrapyrazoles DuP 937 and DuP 941 (Losoxantrone) in the National Cancer Institute preclinical antitumor drug discovery screen. *Journal of the National Cancer Institute*. 1994; 86: 1239–1244.
7. Van Wyk B, Van Heerden F, Van Oudtshoorn B. *Poisonous Plants of South Africa.* Briza Publications, 2002.
8. Cragg G.M., Newman D.J. and Stringner S.Y., Natural Product Extracts of Plant and Marine Origin Having Anti-leukemia potential. The NCI Experience. *Journal of Natural Products* 2005; 69: 488-498.
9. Lee IS, Shamon LA, Kinghorn AD, et al. Cell-cycle specific cytotoxicity mediated by rearranged *ent*-kaurene diterpenoids isolated from *Parinari Curatellifolia. Chem Biol Interact* 1996; 99: 193-204.
10. Nguyen AT, Malonne H, Duez P, et al. Cytotoxic constituents from *Plumbago zeylanica. Fitoterapia* 2004; 75: 500-504.