Journal of Ethnopharmacology 119 (2008) 455–461

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

In vitro anticancer screening of South African plants

G. Fouche[a,*], G.M. Cragg[b], P. Pillay[a], N. Kolesnikova[a], V.J. Maharaj[a], J. Senabe[a]

[a] *Biosciences, Council for Scientific and Industrial Research, PO Box 395, Pretoria, Gauteng 0001, South Africa*
[b] *Natural Products Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, NCI-Frederick, MD 21702-1201, USA*

ARTICLE INFO

Article history:
Received 29 April 2008
Received in revised form 24 June 2008
Accepted 9 July 2008
Available online 16 July 2008

Keywords:
Anticancer
Asteraceae
Medicinal plants
Natural products
Plants
South Africa

ABSTRACT

Aim of the study: The purpose of the present study is to evaluate South African plants for their anticancer activity.
Materials and methods: Plant species were collected throughout South Africa and voucher specimens were deposited and identified at the South African National Biodiversity Institute. Plant extracts were prepared and screened for *in vitro* anticancer activity against a panel of three human cell lines (breast MCF7, renal TK10 and melanoma UACC62) at the CSIR. Plant extracts that exhibited anticancer activity against these three human cell lines were screened by the NCI against sixty human cancer cell lines organized into sub-panels representing leukaemia, melanoma, cancer of the lung, colon, kidney, ovary, central nervous system, breast and prostate.
Results: A total of 7500 plant extracts were screened for *in vitro* anticancer activity against breast MCF7, renal TK10 and melanoma UACC62 human cell lines between the period 1999 and 2006. Hits were classified into four categories based on their total growth inhibition of the cell lines. A hit rate of 5.9% was obtained for extracts which showed moderate activity and these were screened by the NCI against a panel of sixty human cancer cell lines. The extracts of plant species with limited published information for their anticancer properties were subjected to bioassay-guided fractionation and the active constituents isolated and identified. The largest number of plant specimens in this study was from the family Asteraceae, which is rich in sesquiterpene lactones.
Conclusions: Although the extracts of the plants were randomly selected, 68% of these plant species which were hits in the screening programme are reported to be used medicinally. Based on our data, it appears that unrelated medicinal use of the source plants may serve as an initial guide to selection of plants for anticancer screening.

1. Introduction

Cancer is one of the most prominent diseases in humans and currently there is considerable scientific and commercial interest in the continuing discovery of new anticancer agents from natural product sources (Kinghorn et al., 2003). 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 (Cragg and Newman, 2005).

South Africa's rich plant biodiversity, with over 20 000 different species, is a great source of interest to the scientific community (Cherry, 2005). In spite of the success of a natural-product approach to anticancer drug discovery, reports on plants used for the treatment of cancer are rare in South Africa (Steenkamp and Gouws, 2006). 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 the identification of potentially new anticancer drug leads.

An in-house anticancer screening technology was implemented at the CSIR in 1999 with a panel of three human cell lines namely, breast MCF7, renal TK10 and melanoma UACC62. Plant extracts that exhibited anticancer activity against these three human cell lines were screened by the NCI against sixty human cancer cell lines organized into sub-panels representing leukaemia, melanoma, cancer of the lung, colon, kidney, ovary, central nervous system, breast and prostate. The extracts of plant species from the anticancer research programme at CSIR which showed potent anticancer activity has been previously reported by the authors (Fouche et al., 2006).

* Corresponding author. Tel.: +27 12 8412664; fax: +27 12 8414790.
E-mail address: gfouche@csir.co.za (G. Fouche).

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doi:10.1016/j.jep.2008.07.005

This article reports on the anticancer screening programme conducted at the CSIR for extracts of plant species which showed moderate anticancer activity with total growth inhibition (TGI) being observed at concentrations of between 6.25 μg/ml and 15 μg/ml for at least two cell lines.

2. Materials and methods

2.1. Plant material

The plant species were collected throughout South Africa. The plant collectors provided an average of three plant part samples

Table 1
Plant extracts exhibiting moderate[a] *in vitro* anticancer activity at the CSIR

Family	Plant species	Voucher number	Plant part	Extraction solvent[b]	TGI (μg/ml)		
					Renal TK10	Breast MCF7	Melanoma UACC62
Anacardiaceae	*Rhus leptodictya* Diels.	BP00002	Leaves	DCM	8.72	55.42	9.83
Anacardiaceae	*Rhus chirindensis* Baker f.	EN00059	Stems	DCM	8.23	28.04	9.07
Anacardiaceae	*Rhus lancea* L. f.	EN00288	Fruits	DCM	15.00	16.66	13.23
Anacardiaceae	*Rhus lancea* L. f.	EN00288	Stems	DCM	14.71	19.12	13.56
Apocynaceae	*Gomphocarpus physocarpus* Schltr.	EN00160	Roots	DCM:MeOH	6.43	20.80	15.00
Apocynaceae	*Acokanthera oppositifolia* (Lam.) Codd	EN00119	Roots	DCM	<0.00	12.50	6.25
Apocynaceae	*Acokanthera oppositifolia* (Lam.) Codd	EN00119	Stems	DCM:MeOH	<0.00	15.00	11.21
Apocynaceae	*Acokanthera oppositifolia* (Lam.) Codd	EN00119	Stems	DCM	<0.00	6.25	7.75
Apocynaceae	*Gomphocarpus fruticosus* Dryand.	EN00187	Roots	DCM:MeOH	6.38	10.66	<0.00
Apocynaceae	*Gomphocarpus fruticosus* Dryand	EN00187	Fruits	DCM:MeOH	6.78	30.20	11.32
Apocynaceae	*Gomphocarpus fruticosus* Dryand.	CN00026	Leaves and stems	MeOH	<0.00	14.78	12.68
Araliaceae	*Cussonia paniculata* E.&Z.	CN00003	Leaves	DCM:MeOH	6.25	55.68	15.00
Asparagaceae	*Asparagus aethiopicus* L.	EN00618	Roots	DCM:MeOH	18.12	15.00	6.25
Asparagaceae	*Asparagus transvaalensis* (Oberm.) Fellingham & N.L. Mey	EN00356	Stems	DCM:MeOH	17.04	14.39	14.35
Asteraceae	*Brachylaena rotundata* S. Moore	BP00019	Leaves	DCM	15.00	25.95	13.12
Asteraceae	*Xanthium strumarium* L.	EN00146	Stems	DCM	11.24	6.92	6.63
Asteraceae	*Arctotis arctotoides* (L.f.) O. Hoffm.	EN00563	Whole plants	DCM:MeOH	12.70	16.81	13.74
Asteraceae	*Artemisia afra* Jacq.	EN00148	Leaves	DCM:MeOH	26.62	15.00	9.73
Asteraceae	*Athrixia elata* Sond.	EN00039	Leaves and seeds	DCM:MeOH	15.00	27.14	13.34
Asteraceae	*Helichrysum nudifolium* Less.	EN00311	Roots	DCM	33.47	14.43	8.43
Asteraceae	*Oncosiphon piluliferum* (L.f.) Kallersjo	EN00579	Whole plants	DCM	15.00	6.85	19.24
Asteraceae	*Schkuhria pinnata* (Lam) Kuntze	EN00134	Stems	DCM:MeOH	15.00	26.68	9.72
Asteraceae	*Tithonia diversifolia* A. Gray	EN00212	Leaves	DCM:MeOH	9.65	12.70	7.86
Asteraceae	*Vernonia staehelinoides* Harv.	EN00323	Leaves	DCM	14.22	25.49	7.53
Bignoniaceae	*Kigelia africana* (Lam.) Benth.	EN00217	Leaves	DCM	42.88	15.00	8.02
Bignoniaceae	*Kigelia africana* (Lam.) Benth	HV00226	Roots	DCM:MeOH	14.90	8.82	< 0.00
Capparaceae	*Cadaba aphylla* (Thunb.) Wild	EN00471	Roots	DCM	10.14	14.89	16.79
Celastraceae	*Gymnosporia tenuispina* (Sond.) Szyszyl.	EN00090	Stems	MeOH	8.23	28.04	9.07
Celastraceae	*Gymnosporia tenuispina* (Sond.) Szyszyl	EN00090	Flowers and leaves	MeOH	8.23	28.04	9.07
Chrysobalanaceae	*Parinari capensis* Harv.	NB00016	Whole plants	DCM	12.38	6.52	8.25
Combretaceae	*Combretum zeyheri* Sond.	EN00262	Leaves	DCM	15.00	28.21	10.33
Convolvulaceae	*Ipomoea cairica* (L.) Sweet	EN00237	Whole plants	DCM:MeOH	15.00	65.34	14.44
Crassulaceae	*Cotyledon cuneata* Thunb.	EN00608	Roots	DCM:MeOH	7.31	25.00	6.25
Crassulaceae	*Kalanchoe paniculata* Harv.	EN00340	Roots	DCM:MeOH	<0.00	13.25	6.51
Crassulaceae	*Kalanchoe thyrsiflora* Harv.	EN00023	Leaves and roots	MeOH	<0.00	15.00	12.98
Dracaenaceae	*Sansevieria pearsonii* N. E. Br.	CS00017	Roots	DCM:MeOH	25.01	7.70	6.25
Fabaceae	*Psoralea pinnata* L.	EN00024	Stems	DCM	29.88	11.77	9.38
Fabaceae	*Erythrina lysistemon* Hutch.	EN00048	Whole plants	DCM:MeOH	16.24	15.00	9.99
Geraniaceae	*Pelargonium acraeum* R.A. Dyer	EN00035	Whole plants	MeOH	67.26	8.92	12.68
Geraniaceae	*Pelargonium acraeum* R.A. Dyer	EN00035	Whole plants	H_2O	82.12	12.74	12.74
Hyacinthaceae	*Drimia robusta* Bak.	EN00022	Whole plants	DCM:MeOH	6.35	23.28	13.75
Iridaceae	*Moraea polystachya* (Thunb.)	BP00054	Whole plants	DCM:MeOH	6.25	29.63	16.64
Rosaceae	*Leucosidea sericea* Eckl. & Zeyh.	EN00972	Leaves	DCM:MeOH	12.46	14.26	42.94
Solanaceae	*Physalis peruviana* L.	EN00297	Whole plants	DCM:MeOH	9.30	24.95	7.25
Solanaceae	*Physalis peruviana* L.	DS00095	Leaves	DCM:MeOH	9.38	35.59	14.07
Solanaceae	*Solanum acanthoideum* E. Mey.	EN00006	Roots	MeOH	13.36	16.74	14.31
Solanaceae	*Solanum panduriforme* E. Mey.	EN00274	Whole plants	DCM:MeOH	14.12	18.30	9.13
Solanaceae	*Solanum tomentosum* L.	EN00433	Stems	H_2O	54.85	14.68	13.21
Solanaceae	*Withania somnifera* (L.) Dun.	EN00680	Leaves	DCM:MeOH	18.87	14.32	7.52

TGI values of etoposide used as a positive control: [renal TK10: 27.00 μg/ml; breast MCF7: >100 μg/ml; melanoma UACC62: 36.20 μg/ml].

[a] CSIR's criteria: Moderate activity: 6.25 μg/ml < TGI < 15 μg/ml for at least two cell lines.

[b] DCM:MeOH (ratio 1:1).

(e.g. leaves and stems, roots) from a terrestrial plant specimen and each part constituted a separate physical sample. Voucher specimens were deposited and identified at the South African National Biodiversity Institute (SANBI).

2.2. Extraction methods

Plant samples were cut into small pieces and dried in an oven at 30–60 °C. 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. The powdered plant material (100–500 g) was sequentially extracted with dichloromethane (DCM), DCM/methanol (MeOH) (1:1), MeOH and purified water. Organic extracts were concentrated using a rotary vacuum evaporator and then further dried in vacuo at ambient temperature for 24 h. The aqueous extracts were concentrated by freeze-drying. All extracts were stored at −20 °C prior to screening.

2.3. In vitro anticancer screen

All extracts and isolated compounds were first assayed in the three cell line panel consisting of renal TK10, breast MCF7 and melanoma UACC62. These human cell lines were obtained from the NCI as part of the collaborative research agreement between the NCI and the CSIR. The primary anticancer assay was performed in-house (CSIR) in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute (Monks et al., 1991; Kuo et al., 1993; Leteurtre et al., 1994). The human cell lines were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 μM L-glutamine. For a screening experiment, the cells were inoculated into 96-well microtiter plates in 100 μl at plating densities ranging from 5000–40 000 cells/well based on cell growth characteristics. After cell inoculation, the microtiter plates were incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, one plate was fixed with TCA to represent a measurement of the cell population for each cell line at the time of extract/compound addition (T_0).

Experimental extracts and compounds were solubilized in dimethylsulfoxide. At the time of extract/compound addition, the concentrate of extract was diluted in RPMI containing 50 mg/ml gentamicin to the desired final maximum test concentration (100 μg/ml) for the single dose assay. All the extracts/compounds, which reduced the growth of two of the cell lines by 75% or more, were further tested at five serial dilution concentrations ranging from 6.25–100 μg/ml for extracts or 10 fold serial dilutions for compounds. Aliquots of 100 μl of these different extract dilutions were added to the appropriate microtiter wells already containing 100 μl of the medium, resulting in the required final extract concentrations (100, 50, 25, 12.5, 6.25 μg/ml in the plates). Cells without extract addition served as control.

After addition of the extract/compound, the cultures were incubated for 48 h at 37 °C, 5% CO_2, 95% air and 100% relative humidity. 50 μl of cold 50% TCA was added to fix the cells. The supernatant was removed and the plates were washed with water and air-dried. Sulforhodamine B (SRB) solution (100 μl) at 0.4% (w/v) in 1% acetic acid was added to each well and plates were incubated for 10 min at room temperature. Plates were stained and unbound dye was removed by washing five times with 1% acetic acid. After washing, the plates were air-dried. 10 μM Trizma base was used to solubilized the stains and the intensity of the absorbed radiation was read on an automated plate reader at a wavelength of 540 nm. The growth percentage was evaluated spectrophotometrically versus controls not treated with test agents. All tests were performed in duplicate and the average values calculated. The TGI was calculated from $(T - T_0)/(C - T_0) \times 100 = 0$; T is the optical density of the test extract/compound; T_0 is the optical density at time zero and C, the optical density of the control. Etoposide was used as a positive control in the experiments (refer to Table 1).

The results of the five dose assays were reported as TGI (total growth inhibition) values. The assay results for the extracts screened were separated into four categories: inactive (TGI > 50 μg/ml), weak activity (15 μg/ml < TGI < 50 μg/ml), moderate activity (6.25 μg/ml < TGI < 15 μg/ml) and potent activity (TGI < 6.25 μg/ml). Extracts from the latter two categories were selected for further *in vitro* testing for selective cytotoxicity against panels of human cancer cell lines at the NCI. The NCI panel of 60 cell lines included leukemia (L) lines [CCRF-CEM, HL-60(TB), K-562, MOLT-4, RPMI-8226], non-small cell lung cancer (NSCLC) lines [A549/ATCC, EKVX, HOP-62, NCI-H226, NCI-H23, NCI-H322M, NCI-H460, NCI-H522], colon cancer (CL) lines [COLO 205, HCT-116, HCT-15, HT29, KM12, SW-620], central nervous system cancer (CNSC) lines [SF-268, SF-295, SF-539, SNB-19, U251], melanoma (M) lines [LOX IMVI, M14, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257, UACC-62], ovarian cancer (OC) lines [IGROV1, OVCAR-3, OVCAR-5, OVCAR-8, SK-OV-3], renal cancer (RC) lines [786-0, A498, ACHN, CAKI-1, SN12C, TK-10, UO-31], prostate cancer (PC) lines [PC-3, DU-145] and breast cancer (BC) lines [MCF7, NCI/ADR-RES, MDA-MB-231/ATCC, HS 578T, MDA-MB-435, MDA-N, BT-549]. Hit rates were calculated based on the number of plant species which showed moderate anticancer activity expressed as a percentage of the total plant species tested.

2.4. Bioassay-guided fractionation and isolation of active compounds

A general separation procedure was followed which involved liquid–liquid partitioning of the organic extract (DCM, DCM/MeOH or MeOH) between methanol–water (90%, v/v) and hexane three times in a separating funnel. The hexane layers were combined and evaporated under reduced pressure and the aqueous residue was diluted with water, concentrated by evaporation of the methanol under reduced pressure and extracted three times with dichloromethane. The combined dichloromethane layers were evaporated under reduced pressure. The water layer was concentrated by evaporating under vacuum and thereafter it was subjected to freeze-drying. The resulting three fractions were tested against the three-cell lines, breast MCF7, renal TK10 and melanoma UACC62 at five serial concentrations ranging from 6.25 to 100 μg/ml. The most active fraction was further subjected to bioassay-guided fractionation using either silica or reversed phase column chromatography. This resulted in the isolation and identification of the active ingredients which were tested at serial dilution concentrations in the five dose assay. All tests were performed in duplicate and average values calculated.

3. Results and discussion

Between late 1998 and 2006, the Bioprospecting group of the CSIR coordinated the collection of approximately 22 000 samples of higher plants (which included multiple parts per single plant specimen) through trained plant collectors. These samples were processed and extracted as described in the materials and methods section. To date 7500 randomly selected plant extracts (representing 700 plant species) were tested in the three-cell line pre-screen, which was performed at a single dose of 100 μg/ml. A total of 950 extracts exhibited a growth inhibition of above 75% for two or more of the cell lines (GI_{75}). This criterion has been applied, because the cell lines (MCF7, TK10 and UACC62) are highly

Table 2
In vitro anticancer activity of plant extracts tested at the NCI

Family	Plant species	Plant part	Extraction solvent[a]	Mean log GI_{50}[b]	TGI (μg/ml) of three most active cell lines
Anacardiaceae	*Rhus leptodictya* Diels.	Leaves	DCM	0.93 M	9.77 (NSCLC NCI-H522); 11.48 (CNSC U251); 14.13 (leukaemia CCRF-CEM)
Anacardiaceae	*Rhus chirindensis* Baker f.	Stems	DCM	0.88 M	2.82 (NSCLC NCI-H522); 11.75 (breast HS 578T); 12.30 (CNSC U251)
Apocynaceae	*Gomphocarpus physocarpus* Schltr.	Roots	DCM:MeOH	0.42 M	2.04 (NSCLC A549/ATCC); 2.40 (prostate DU-145); 2.69 (renal RXF 393)
Apocynaceae	*Gomphocarpus fruticosus* Dryand.	Roots	DCM:MeOH	0.61 M	3.72 (renal RXF 393); 3.72 (ovarian OVCAR-3); 5.01 (renal TK-10)
Araliaceae	*Cussonia paniculata* E.&Z.	Leaves	DCM:MeOH	0.45 M	1.00 (leukaemia RPMI-8226); 1.45 (colon HCT-116); 2.69 (colon KM12)
Asteraceae	*Brachylaena rotundata* S. Moore	Leaves	DCM	1.48 W	12.30 (leukaemia SR); 19.95 (ovarian OVCAR-5); 24.55 (colon HT29)
Asteraceae	*Xanthium strumarium* L.	Stems	DCM	0.82 M	8.91 (breast MCF7); 8.91 (colon HCT-116); 9.12 (renal 786-0)
Asteraceae	*Artemisia afra* Jacq.	Leaves	DCM:MeOH	1.02 M	13.49 (NSCLC NCI-H522); 13.49 (melanoma SK-MEL-5); 14.13 (colon HT29)
Asteraceae	*Athrixia elata* Sond.	Leaves and seeds	DCM:MeOH	0.94 M	12.88 (prostate PC-3); 13.49 (CNSC U251); 13.49 (colon HCT-15)
Asteraceae	*Schkuhria pinnata* (Lam) Kuntze	Stems	DCM:MeOH	0.84 M	2.88 (NSCLC NCI-H522); 4.07 (colon COLO 205); 4.57 (colon HCT_116)
Bignoniaceae	*Kigelia africana* (Lam.) Benth.	Leaves	DCM	1.22 W	12.30 (breast BT-549); 9.55 (melanoma MALME-3M); 19.95 (melanoma M14)
Celastraceae	*Gymnosporia tenuispina* (Sond.) Szyszyl.	Stems	MeOH	0.80 M	4.68 (breast T-47D); 4.68 (leukaemia SR); 6.31 (leukaemia K-562)
Celastraceae	*Gymnosporia tenuispina* (Sond.) Szyszyl	Flowers and leaves	MeOH	−0.54 P	0.06 (leukaemia CCRF-CEM); 0.10 (leukaemia MOLT-4); 0.28 (leukaemia SR)
Crassulaceae	*Kalanchoe thyrsiflora* Harv.	Leaves and roots	MeOH	0.34 M	1.00 (CNSC SF-539); 1.86 (NSCLC A549/ATCC); 2.09 (NSCLC HOP-62)
Dracaenaceae	*Sansevieria pearsonii* N. E. Br.	Roots	DCM:MeOH	1.31 W	28.84 (NSCLC NCI-H322M); 29.51 (CNSC U251); 29.51 (melanoma M14)
Fabaceae	*Psoralea pinnata* L.	Stems	DCM	0.83 M	2.40 (NSCLC NCI-H522); 11.22 (renal UO-31); 8,71 (renal ACHN)
Fabaceae	*Erythrina lysistemon* Hutch.	Whole plants	DCM:MeOH	0.64 M	4.37 (CNSC SF-539); 6.31 (leukaemia HL-60(TB)); 6.46 (NSCLC NCI-H460)
Geraniaceae	*Pelargonium acraeum* R.A. Dyer	Whole plants	MeOH	0.91 M	8.91 (melanoma UACC-62); 10.23 (renal RXF 393); 10.72 (renal A498)
Geraniaceae	*Pelargonium acraeum* R.A. Dyer	Whole plants	H_2O	0.93 M	1.07 (renal A498); 6.92 (ovarian OVCAR-3); 7.41 (renal RXF 393)
Hyacinthaceae	*Drimia robusta* Bak.	Whole plants	DCM:MeOH	0.08 M	1.05 (ovarian OVCAR-3); 1.38 (CNSC SF-539); 1.41 (NSCLC A549/ATCC)
Solanaceae	*Physalis peruviana* L.	Whole plants	DCM:MeOH	0.35 M	0.27 (prostate PC-3); 1.07 (leukaemia RPMI-8226); 4.37 (leukaemia SR)
Solanaceae	*Solanum acanthoideum* E. Mey.	Roots	MeOH	1.33 W	16.22 (renal 786-0); 18.62 (renal SN12C); 18.62 (ovarian IGROV1)
Verbenaceae	*Lippia javanica* (Burm. f.) Spreng	Roots	DCM	0.87 M	1.82 (breast MDA-MB-435); 1.86 (breast MDA-N); 2.09 (melanoma MALME-3M)

NSCLC: non-small cell lung cancer; CNSC: central nervous system cancer.
[a] DCM:MeOH (ratio 1:1).
[b] NCI's criteria: W, weak activity: log GI_{50} > 1.10–1.5; M, moderate activity: log GI_{50} > 0–1.10; P, potent activity: log GI_{50} < 0.

sensitive to detect anticancer activity. The plant species which showed moderate anticancer activity after screening all 950 extracts at five serial dilution concentrations ranging from 6.25 to 100 μg/ml are shown in Table 1. A selected number of these moderately active extracts were tested at the NCI (see Table 2) 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.

The 50 plant extracts which showed moderate anticancer activity listed in Table 1, represent 20 phyla and 41 plant species. A hit rate of 5.9% was obtained based on the number of species with moderate activity expressed as a percentage of the 700 plant species tested. The majority of aqueous extracts did not show any activity in the screen.

Desktop literature investigations aimed at establishing information on the use of the plants as anticancer treatments were conducted prior to any attempts to isolate the active ingredients. The extracts of plant species with limited published information for their anticancer properties were subjected to bioassay-guided fractionation and the active constituents were isolated and identified (Fig. 1).

Among the 50 moderate extracts, 7 belong to the family Apocynaceae, representing three plant specimens and a hit rate of 0.4%. The plant genera, *Acokanthera* and *Gomphocarpus* are sources of toxic cardiac glycosides and are well-known throughout Africa as a traditional source of extremely toxic arrow poisons (Van Wyk and Gericke, 2000) and due to this were not fractionated and further investigated. High hit rates were also observed for the family, Apocynaceae by the NCI, but this was attributed to other genera tested containing indole alkaloids for example, *Cathanthus roseus* (Cragg et al., 2005).

Three plant species, *Kalanchoe paniculata*, *Kalanchoe thyrsiflora* and *Cotyledon cuneata* (0.4% hit rate) of the family Crassulaceae, are reported to contain bufadienolides and these are toxic to livestock and cause the well-known poisoning syndrome known as nenta or krimpsiekte (Van Wyk et al., 2002). These plant species were also not further investigated. It is interesting to note, that the organic extracts of the plants containing cardiac glycosides, *Acokanthera*

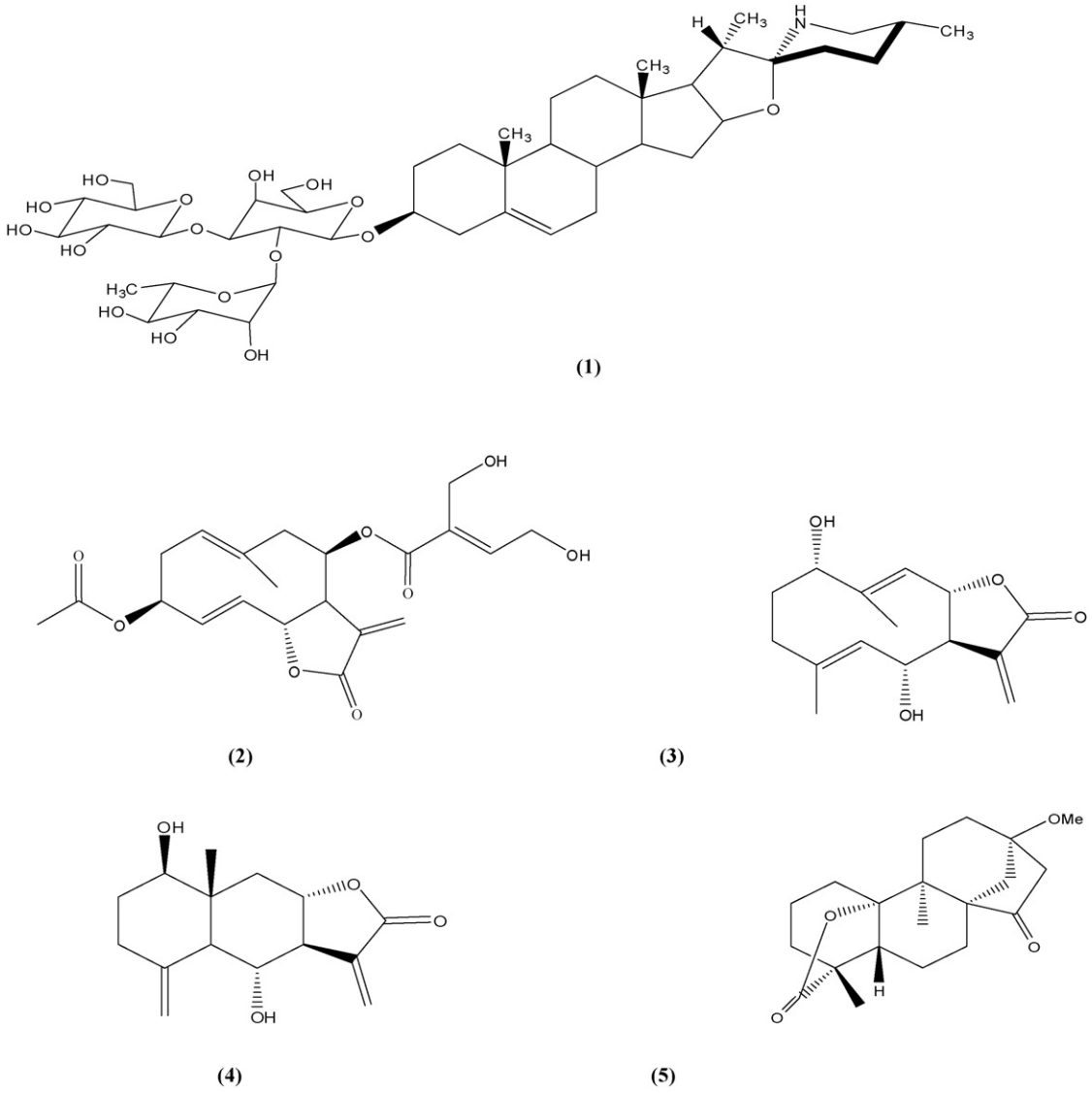

Fig. 1. Structures of constituents isolated.

oppositifolia, *Kalanchoe paniculata* and *Kalanchoe thyrsiflora* did not exhibit a general cytotoxicity against all three cell lines, but displayed potent activity against only one of the cell lines, namely renal (TK10) whilst moderate activity was observed for the other two-cell lines.

The Solanaceae family, representing five plant species (0.7% hit rate) is a source of steroidal alkaloids and bioassay-guided fractionation of *Solanum panduriforme* and *Solanum aculeatissimum* yielded solasonine (**1**) [TGI for TK10 (4.60 μg/ml), MCF7 (3.77 μg/ml) and UACC62 (3.08 μg/ml] with reported cytotoxicity and cancer-related activity (Cragg et al., 2005). The largest number of plant specimens in this study was from the family Asteraceae, representing 10 plant species (1.4% hit rate). This family also represented the highest hit rate for plant species with potent activity (Fouche et al., 2006). This family is rich in sesquiterpene lactones and eucannabinolide (**2**) was isolated as the active constituent from the stems of *Schkuhria pinnata*. Eucannabinolide displayed the highest activity against the melanoma UACC62 cell line (TGI < 6.25 μg/ml). The total growth inhibition for the breast MCF7 and renal TK10 cancer cell lines were 7.25 μg/ml and 12.00 μg/ml, respectively. This sesquiterpene lactone was also isolated from a related species of *Schkuhria* namely, *Schkuhria virgata* and exhibited *in vivo* antileukemic activity (Herz and Govindan, 1980).

Fractionation of the dichloromethane extract of the whole plant of *Oncosiphon piluliferum* yielded two active constituents, namely tatridin A (**3**) [TGI for TK10 (4.50 μg/ml), MCF7 (86.10 μg/ml) and

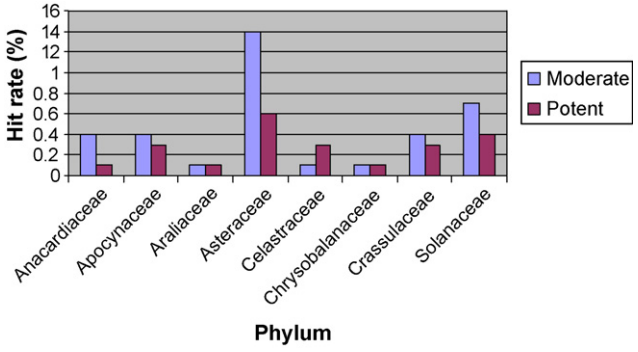

Fig. 2. Comparison of the plant species showing potent and moderate activity expressed as a percentage of the total number tested.

Table 3
Reported traditional uses of plant species identified to have moderate anticancer activity

Plant species	Traditional use
Acokanthera oppositifolia (Lam.) Codd	The dried leaves or roots are used to treat headaches or as a treatment for snakebite. Other ailments treated include toothache, colds, anthrax and tapeworm. *Acokanthera* species are best known as sources of extremely toxic arrow poisons (Van Wyk et al., 1997)
Artemisia afra Jacq.	The stems and leaves are used for fever, colds, flu, sore throat, coughs, asthma and pneumonia (Van Wyk and Gericke, 2000). Vhavenda use leaf infusions as anthelmintics for respiratory ailments and as prophylactics against dysentery, diarrhoea and malaria (Mabogo, 1990)
Athrixia elata Sond.	The leaf and root are a Southern Sotho remedy for sore feet and is used as a tea. The Tswana administer a decoction of the herb as a stimulant to a convalescent (Watt and Breyer-Brandwijk, 1996)
Cadaba aphylla (Thunb.) Wild	The moist powdered plant is applied as a poultice between layers of gauze to draw boils and abscesses. The root is used in small doses as a tonic and also as a purgative (Van Wyk and Gericke, 2000)
Combretum zeyheri Sond.	Bark is used for gallstones. Root infusions are taken for bloody diarrhoea in Zimbabwe (Hutchings et al., 1996)
Cussonia paniculata E.&Z.	The thick tuberous roots are peeled and eaten raw as an emergency food or as a source of water (Van Wyk and Gericke, 2000)
Drimia robusta Bak.	Bulbs are used as expectorants, emetics, diuretics and heart tonics (Van Wyk and Gericke, 2000)
Erythrina lysistemon Hutch.	Bark is used for swellings and abscesses. Vhavenda use bark for toothache (Hutchings et al., 1996)
Gomphocarpus fruticosus Dryand.	Leaf infusions are administered for diarrhoea and stomach pain in children. Also used as snuffs and in emetics taken to strengthen the body (Hutchings et al., 1996)
Helichrysum nudifolium Less.	Roots decoctions are taken sometimes as emetics for chest complaints. Unspecified parts are reported to be used for colds (Watt and Breyer-Brandwijk, 1996). In unspecified parts of South Africa roots are used for coughs and colds while leaves are used for dress wounds (Hutchings et al., 1996)
Ipomoea cairica (L.) Sweet	Leaves are traditionally used as abortifacients in Nigeria. Infusions from a double handful of leaves crushed in a cupful of cold water are taken for relief of febrile rashes (Hutchings et al., 1996)
Kalanchoe paniculata Harv.	Ash from leaves and flowers used by men as a love charm. Causing poisoning in stock (Hutchings et al., 1996)
Kalanchoe thyrsiflora Harv.	Roots decoctions are used as anthelmintic enemas and are administered to pregnant women who do not feel well (Watt and Breyer-Brandwijk, 1996)
Leucosidea sericea Eckl. & Zeyh.	Ground leaf paste is applied to the eyeball and inside and outside the lids for ophthalmia (Watt and Breyer-Brandwijk, 1996)
Kigelia africana (Lam.) Benth.	Powdered fruit is used as a dressing for ulcers and also for syphilis and rheumatism. Roots are used for gynaecological complaints, constipation and tapeworm (Hutchings et al., 1996)
Lippia javanica (Burm. f.) Spreng	The Vhavenda use leaf infusions as anthelmintics for respiratory ailments and as prophylactics against dysentery, diarrhoea and malaria (Mabogo, 1990)
Oncosiphon piluliferum (L.f.) Kallersjo	Leaf paste is used for skin complaints (Hutchings et al., 1996)
Parinari capensis Harv.	This plant is mixed with another medicine to facilitate conception in women. The fruit is edible and used to make beer (Watt and Breyer-Brandwijk, 1996)
Physalis peruviana L.	Leaves and roots have been used as diuretics (Watt and Breyer-Brandwijk, 1996). Root decoctions are used for infantile colds, skin rashes, excess bile, labour pains, gonorrhoea and general ill-health (Kokwaro, 1976)
Psoralea pinnata L.	Taken as emetics by healers with mental disturbances associated with their calling (Hutchings et al., 1996)
Rhus chirindensis Baker f.	The plant is used for heart complaints. The bark is used to strengthen the body, stimulate circulation and for rheumatism (Hutchings et al., 1996)
Rhus lancea L. f.	The wood of *Rhus lancea* is used by the Thiaping for tanning (Watt and Breyer-Brandwijk, 1996)
Solanum acanthoideum E. Mey.	Topically applied for ringworm. Burnt fruit is rubbed into scarifications over painful parts and for the relief of rheumatism (Watt and Breyer-Brandwijk, 1996)
Solanum panduriforme E. Mey.	Sap of fruit is used for skin infections. Roots are used for toothache and root decoctions are taken for haemorrhoids (Hutchings et al., 1996)
Solanum tomentosum L.	A decoction of the root is a Zulu remedy for syphilis. The Southern Sotho uses the plant as a medicine for sore throat. The juice from both unripe and ripe fruit has given negative toxicity tests (Watt and Breyer-Brandwijk, 1996)
Tithonia diversifolia A. Gray	For stomach pains, indigestion, sore throat and liver pains grind the leaves put them into water and drink the infusion (Kokwaro, 1976)
Withania somnifera (L.) Dun.	In Southern Africa the plant is taken for fever, colds and flu, asthma, abdominal discomfort, diarrhoea, worms and as a sedative and hypnotic (Van Wyk and Gericke, 2000)
Xanthium strumarium L.	The plant is said to be used as a diaphoretic and sedative. In Assam the young flowering top and the first two leaves are eaten as a pot herb. In Europe, the leaf, fruit and root have been used as remedies for catarrh, scofula, leprosy, tubercular and other skin conditions, cancer, dysenteries and bladder ailments and as a diaphoretic (Watt and Breyer-Brandwijk, 1996)

UACC62 (18.72 μg/ml] and deacetyl-β-cyclopyrethrosin (**4**) [TGI for TK10 (5.89 μg/ml), MCF7 (86.15 μg/ml) and UACC62 (16.92 μg/ml]. Recently, new strategies were employed to develop analogues of a naturally occurring sesquiterpene lactone, parthenolide, to improve its water solubility as an antileukemic agent (Jordan, 2007).

A cytotoxic *ent*-kaurene diterpenoid, 13-methoxy-15-oxozoapatlin (**5**) [TGI of renal and breast cell line were less than 6.25 μg/ml; melanoma (9.14 μg/ml)] was isolated from the bioassay-guided fractionation of *Parinari curatellifolia* and *Parinari capensis* (Fouche et al., 2006). The structure and cytotoxicity was published by Lee et al. (1996) and the compound showed selectivity for leukaemia cell lines.

The average activity (mean log GI_{50}) of the extracts tested at the NCI correlated well with that tested at the CSIR and is shown in Table 2. From the 23 extracts tested, 18 exhibited moderate activity

in both screens. The organic extract of *Physalis peruviana* showed potent and selective activity against the prostate PC-3 cancer cell line (TGI of 0.27 μg/ml) at the NCI and might be a good hit to further investigate. The methanolic extract of the flowers and leaves of *Gymnosporia tenuispina* showed potent activity in the NCI 60 cell line assay and selectivity was observed for three leukaemia cell lines, namely CCRF-CEM, MOLT-4 and SR. It might be worthwhile to research this plant for the treatment of leukaemia. Furthermore, the extract of the stems of *Gymnosporia tenuispina* exhibited moderate activity at the NCI versus the potent activity observed for the extract of the flowers and leaves of *Gymnosporia tenuispina*, indicating the presence of active constituents in these parts of the plant.

The data for the plant species showing moderate activity compare well with that of the plant species having potent activity and are summarised in Fig. 2. From this data only the phylum Celastraceae shows a higher hit rate for plant species having potent activity and might be a possible source for potential anticancer agents.

Although the plants selected were randomly chosen, 68% of the hits were reported to be used medicinally. The recorded medicinal uses of the plant species are shown in Table 3. 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 (Fouche et al., 2006).

4. Conclusions

A total of 7500 plant extracts were screened for *in vitro* anticancer activity against a panel of three human cell lines and the results showed that the largest number of plant specimens in this study was from the family Asteraceae, which is rich in sesquiterpene lactones. Although the extracts of the plants were randomly selected, the majority of these plant species were reported to be used medicinally. Based on our data, it appears that unrelated medicinal use of the source plants may serve as an initial guide to selection of taxa for anticancer screening. The most prominent discovery in this respect was that of the highly effective anticancer agents, vinblastine and vincristine, which were isolated from *Catharanthus roseus* and used by various cultures for the treatment of diabetes (Gueritte and Fahy, 2005). Plants still remain a prime source of drugs for the treatment of cancer and can provide leads for the development of novel anticancer agents.

Acknowledgements

The authors would like to thank the South African National Biodiversity Institute (SANBI) for the identification of plant specimens and the U.S. National Cancer Institute (NCI) for 60 human cell line testing. This work was financially supported by the Council for Scientific and Industrial Research.

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