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South African Journal of Botany 72 (2006) 630–633
SOUTH AFRICAN JOURNAL OF BOTANY
www.elsevier.com/locate/sajb

Short communication

Cytotoxicity of six South African medicinal plant extracts used in the treatment of cancer

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Received 25 October 2005; accepted 23 February 2006

Abstract

Aqueous extracts prepared from six South African medicinal plants, with cancer-related ethnobotanical uses, were tested for their cytotoxic ability *in vitro* against three human cancer cell lines: DU-145 prostate cancer cells, MDA-MB-231 and MCF-7 breast cancer cells and a non-malignant breast cell line, MCF-12A. The plants studied were: *Bidens pilosa*, *Centella asiatica*, *Cnicus benedictus*, *Dicoma capensis*, *Hypoxis hemerocallidea* and *Sutherlandia frutescens*. Of these plants, only *D. capensis* exhibited pronounced cytotoxic effects in two of the cell lines tested: MCF-7 and MCF-12A.

Keywords: Cancer; Cytotoxity; Medicinal plants; South Africa

1. Introduction

Reports on plants used for the treatment of cancer are rare in South Africa. Mongelli et al. (2000) found this to be true for Argentina plants, and ascribed it to the fact that cancer involves a complex set of signs and symptoms. It has been recommended that ethnopharmacological usages such as immune and skin disorders, inflammatory, infectious, parasitic and viral diseases be taken into account when selecting plants used to treat cancer, since these reflect disease states bearing relevance to cancer or a cancer symptom (Cordell et al., 1991; Popoca et al., 1998).

Since the majority of cancer chemotherapeutants severely affect the hosts' normal cells (Mascarenhas, 1994), the use of natural products has now been contemplated of exceptional value in the control of cancer (Suffness and Pezzuto, 1990). Furthermore, the search for new sources of biologically active compounds is important for the discovery of new drugs for the treatment of cancer.

This study determined the cytotoxic activities in aqueous extracts of six plants used by traditional healers in South Africa to treat cancer. The plants investigated were: (i) leaves and stems of *Bidens pilosa* L. (Asteraceae, Mai-Mai market, Johannesburg) used in the treatment of prostate gland tumours and inflammation (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996); (ii) *Centella asiatica* (L.) Urb. (Araliaceae, South African National Biodiversity Institute (SANBI), Tshwane) leaves which are prescribed for skin complaints, rheumatoid arthritis, cancer and fevers (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996); (iii) decoctions of *Cnicus benedictus* L. (Asteraceae, SANBI, Tshwane) taken for the treatment of internal cancer (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 1997); (iv) leaves and twigs of *Dicoma capensis* Less. (Asteraceae, gift from Prof. B-E van Wyk, Department of Botany, Rand Afrikaans University) which are prescribed in cases of cancer, high blood pressure and fever (Van Wyk et al., 1997), (v) *Hypoxis hemerocallidea* Fisch. and C.A. Mey. (Hypoxidaceae, Mai-Mai market, Johannesburg) corms used to treat bladder disorders and testicular tumours (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 1997) and (vi) leaf decoctions of *Sutherlandia frutescens* (L.) R.Br. (Fabaceae, provided by Dr. C. Albrecht, Medical Research Council) which are taken for stomach problems, internal cancer, inflammation and viral diseases (Watt and Breyer-Brandwijk, 1962; Rood, 1994; Van Wyk et al., 1997). Cytotoxicity was determined against a prostate carcinoma cell line (DU-145), breast cancer cell lines (MCF-7 and MDA-MB-231) and a non-malignant breast cell line (MCF-12A).

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doi:10.1016/j.sajb.2006.02.004

All the remedies investigated in this study are prescribed as infusions prepared by traditional healers, therefore, only aqueous extracts were tested *in vitro*. Dried plant material (1 g) was suspended in 10 ml deionised water and brewed as a tea by boiling for 15 min. Extracts were allowed to cool, centrifuged and the supernatants passed through 0.45 μm and 0.22 μm filters, consecutively. The yields were 0.75%, 2.85%, 2.49%, 0.55%, 3.69% and 2.38% for *B. pilosa*, *C. asiatica*, *C. benedictus*, *D. capensis*, *H. hemerocallidea* and *S. frutescens*, respectively.

Human DU-145 prostate carcinoma cells were maintained in Ham's F10 nutrient mixture supplemented with 5% decomplemented foetal calf serum (FCS), gentamycin sulphate (0.004%), glucose (0.57%) and $NaHCO_3$ (0.12%). MCF-12A cells were cultured in a 1:1 mixture of Dulbecco's minimum essential medium (DMEM) and Ham's F12 medium supplemented with epidermal growth factor (20 ng/ml), cholera toxin (100 ng/ml), insulin (10 :g/ml), hydrocortisone (500 ng/ml) and FCS (10%). MDA-MB-231 and MCF-7 breast carcinoma cells were grown in DMEM supplemented with sodium pyruvate (111 mg/l), sodium bicarbonate (2.25 g/l) and 10% FCS.

Cells were seeded into 96-well flat-bottomed plates at a concentration of 3.0×10^5 cells per ml. After 24 h, cells were treated with plant extract, which was diluted with culture medium to a final concentration of 50 μg/ml (Zee-Cheng, 1997; Itharat et al., 2004). XTT labelling reagent (50 :l) was added and the absorbance (560 nm) read after 72 h (Gerlier and Thomasset, 1986). Experiments were carried out three times in triplicate. Active extracts were considered as those with less than 50% survival after an exposure time of 72 h. Cisplatin, a known anti-tumour agent, was used as positive control.

The effects of the plant extracts on proliferation in DU-145, MDA-MB-231, MCF-7 and MCF-12A cells are presented in Fig. 1A–D, respectively. Results showed *B. pilosa* to inhibit proliferation by 10% in the carcinoma cell lines. Inhibition of proliferation of leukemia cells *in vitro* has been reported with hot water extracts of this plant (Chang et al., 2001). In four of the five leukemia cell lines tested by these authors, the IC_{50} ranged between 145 and 200 μg/ml, concentrations 3–4 fold above that tested in the present study. There are reports that describe *B. pilosa* as having antiviral (Chiang et al., 2003), anti-leukemic (Chang et al., 2001), anti-bacterial (Rabe and van Staden, 1997), anti-malarial (Brandao et al., 1997), anti-oxidative (Chiang et al., 2004), immunosuppressive and anti-inflammatory activity (Jäger et al., 1996; Pereira et al., 1999). The extracts of *H. hemerocallidea* stimulated DU-145 and MCF-12A cell growth and inhibited the growth of the MCF-7 cells. This plant has been reported to display anti-inflammatory activity (Ojewole, 2002), an activity related to cancer. The aqueous extracts of *S. frutescens* inhibited growth of the oestrogen dependent cancer cell lines and stimulated the growth of the MCF-12A and MDA-MB-231 cells. Chinkwo (2005) found the crude aqueous whole plant extract of *S. frutescens* to induce cytotoxicity in cervical carcinoma and Chinese Hamster Ovary cells. Ethanolic extracts of *S. frutescens* commercial preparations (tablets and powder) have been reported to inhibit proliferation of both MCF-7 and MDA-MB-468 human breast

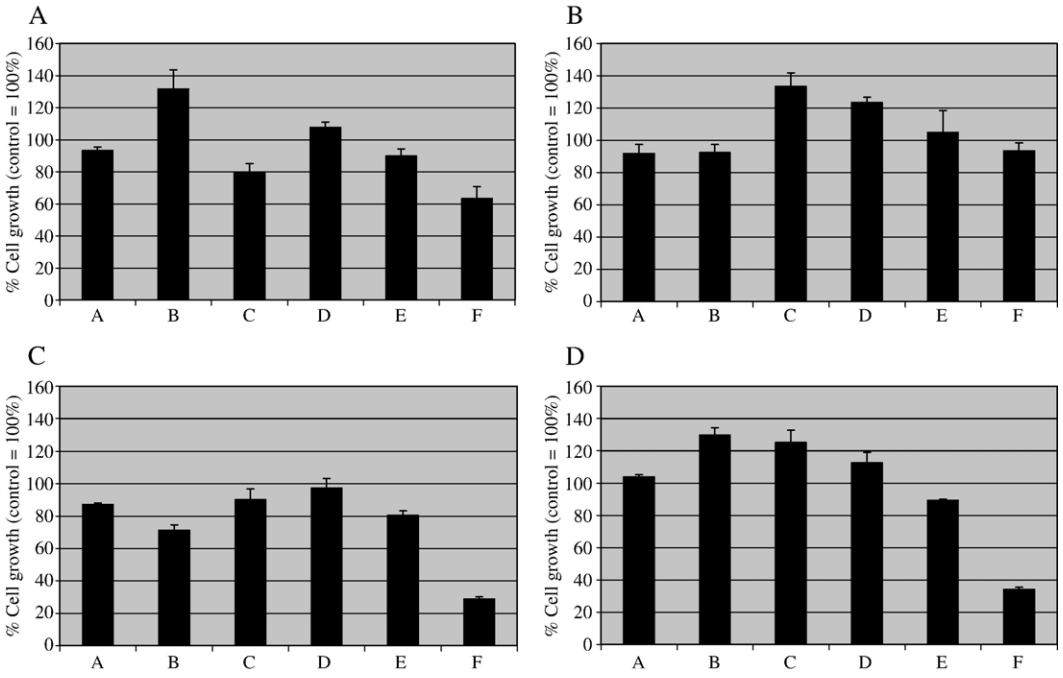

Fig. 1. The effect of 50 μg/ml plant extract on (A) DU-145 prostate cancer cells, (B) MDA-MB-231 breast cancer cells, (C) MCF-7 breast cancer cells and (D) MCF-12A non-malignant breast cells after an exposure time of 72 h; (A) *Bidens pilosa*; (B) *Hypoxis hemerocallidea*; (C) *Sutherlandia frutescens*; (D) *Centella asiatica*; (E) *Cnicus benedictus*; (F) *Dicoma capensis*. The values are expressed as means ± S.E.M. ($n = 3$).

cancer cells, human leukemia Jurkat cells, human promyelocyte HL60 cells and murine RAW 264.7 macrophage/monocyte cells (Tai et al., 2004). *S. frutescens* has been shown to possess antioxidant potential (Fernandes et al., 2004), which is thought to account for some of the anti-inflammatory properties already known (Ojewole, 2004). In this study extracts of *C. asiatica* stimulated the growth of three of the cell lines tested. Crude and partially purified fractions of a methanolic extract of *C. asiatica* have been reported to inhibit proliferation of transformed cell lines (Ehrlich ascites tumour cells: IC_{50} 62 μg/ml, Dalton's lymphoma ascites tumour cells: IC_{50} 75 μg/ml), and to be non-toxic to normal human lymphocytes (Babu et al., 1995). Antifungal, antibacterial, anti-inflammatory and anti-allergic activity has been described for *C. asiatica* (Ponglux et al., 1987). *C. benedictus* and *D. capensis* inhibited the proliferation of DU-145, MCF-7 and MCF-12A cells. We found no literature where the cytotoxic activity of *C. benedictus* or *D. capensis* has previously been investigated.

The American National Cancer Institute guidelines set the limit of activity for crude extracts at a 50% inhibition (IC_{50}) of proliferation of less than 30 μg/ml after an exposure time of 72 h (Suffness and Pezzuto, 1990). With the exception of *D. capensis*, the other plant species had IC_{50} values higher than 50 μg/ml. The IC_{50} values of the latter plant were 30 μg/ml and 31 μg/ml in MCF-7 and MCF-12A cells, respectively. The positive control, cisplatin, had IC_{50} values of 0.27 μg/ml and 0.14 μg/ml in MCF-7 and MCF-12A cells, respectively.

Some of the remedies investigated in this study have been studied from a chemical point of view. The active substances of *H. hemerocallidea* (rooperol), *C. benedictus* (cnicin, arctigenin, arctiin) and *S. frutescens* (canavanine) are known to exhibit tumouricidal or cytotoxic activity (Nicoletti et al., 1992; Hirano et al., 1994; Southon, 1994; Swaffar et al., 1994; Moritani et al., 1996). *B. pilosa* contains phenylheptatriyene which amongst others kills human fibroblast cells (Morton, 1962). Asiaticoside isolated from *C. asiatica* is reported to possess an IC_{50} of 1.58 ±0.15 mg/ml in MCF-7 cells (Huang et al., 2004).

Since cytotoxicity of the plants investigated has been reported by other authors, it is evident that different cell lines exhibit different sensitivities towards the plant extracts. Also, some plants are reported to have a cytotoxic effect on cancer cells (Kusuge et al., 1985; Alley et al., 1988) whereas other plant extracts activate several parameters of the immune system as a strategy to destroy cancer (Abuharfeil et al., 2000). Differences in results obtained in this study and those reported in the literature could also be ascribed to differences in extraction procedures and the natural variability in plants.

Since *D. capensis* showed the most pronounced cytotoxic activity, this plant will be evaluated further for the possible isolation of active compounds.

Acknowledgements

The Cancer Association of South Africa, Struwig-Germeshuysen and the Wolmarans Research Trust are thanked for financial support.

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Edited by P.J. Houghton