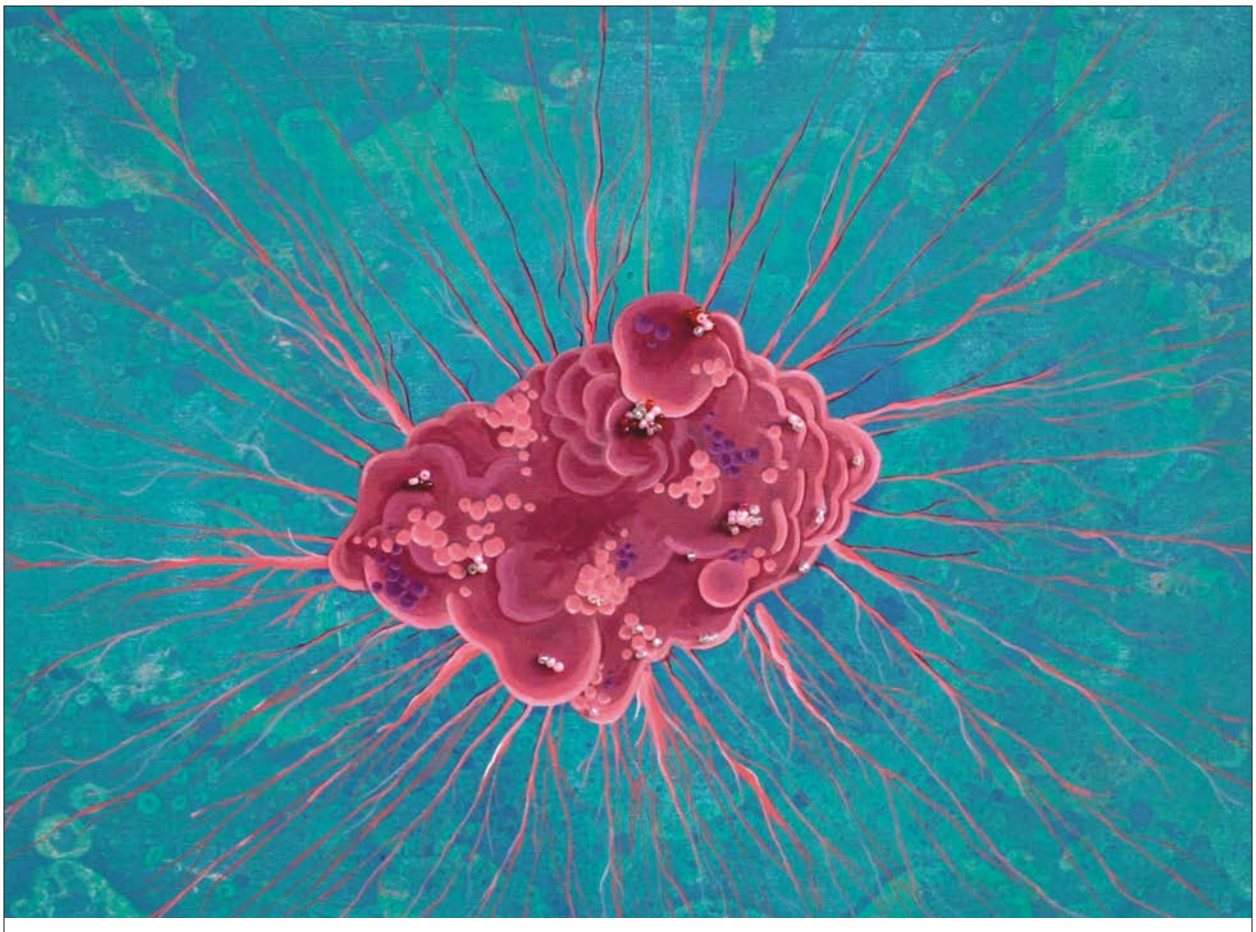

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In vitro Antitumour Activity of *Solanum aculeastrum* Berries on Three Carcinoma Cells

[1]S. Koduru, [1]D.S. Grierson, [2]M. van de Venter and [1]A.J. Afolayan
[1]Department of Botany, University of Fort Hare, Alice 5700, South Africa
[2]Department of Microbiology and Biochemistry, Nelson Mandela Metropolitan University, Port Elizabeth 6031, South Africa

Abstract: *Solanum aculeastrum* is a medicinal plant used by the traditional healers of the Eastern Cape of South Africa, for the treatment of cancers. The antiproliferative activities of this plant were studied *in vitro* using three human tumour cell lines (HeLa, MCF7 and HT29). Methanolic extracts of the fruits had the highest antiproliferative activity with IC_{50} between 17.1 and 41.9 μg mL^{-1} while the activities of their aqueous extracts ranged between 27.9 and 48.5 μg mL^{-1}. The leaf extracts had no anticancer activity under the experimental conditions tested. Overall, the HeLa and MCF7 cell lines were much more sensitive to both extracts than HT29 cells.

Key words: Traditional medicine, *Solanum aculeastrum*, antitumour, anticancer, cytotoxicity

Introduction

Over one million people are diagnosed annually with breast cancer which is one of the primary causes of deaths among women globally (Ferlay *et al*., 2001). The rate of increase of cancer incidence and lack of anticancer drugs has forced scientists to pharmacological and chemical investigations of medicinal plants in search for anticancer agents. The results of the screening of plant extracts for anti proliferative activity have shown that higher plants are a potential source of antioncogenic agents which can compete favourably with chemotherapy and hormonal treatments (Pezzuto, 1997; Wu *et al*., 2002).

Solanum aculeastrum Dunal (Solanaceae) is widely used in traditional medicine for the treatment of human and livestock diseases (Hutchings *et al*., 1996). Both fresh and boiled berries of the plant are used as a cure for jigger wounds and gonorrhea (Agnew and Agnew, 1994). Antimicrobial and antioxidant activity of *Solanum aculeastrum* using crude extracts has been previously reported (Koduru *et al*., 2006a, b). Also, ethnomedical information from the indigenous people of the Eastern Cape Province of South Africa revealed that this plant is used for the treatment of breast cancer (Koduru *et al*., 2006c, d). There is, however, no report on the anticancer property of *S. aculeastrum* in the literature. Yet, species in the genus *Solanum* are known to be rich in steroidal alkaloids and flavonoids which are known to induce apoptosis in tumor cell lines (Papamichael, 2000; Esteves-Souza *et al*., 2002). Indeed, phytochemical investigations of *S. aculeastrum* have revealed the presence of steroidal alkaloids such as solaculine A, solamargine, β-solamarine, solasonine and solasodine (Drewes and Van Staden, 1995; Wanyonyi *et al*., 2002).

In vitro studies have provided evidence that chemotherapeutic agents such as plant extracts may induce apoptotic tumour cell deaths *in vivo*. In this investigation, the *in vitro* cytotoxic properties of the crude extracts of leaves and berries of *S. aculeastrum* was tested against three cancerous cell lines viz. HeLa, HT29 and MCF7 using standard procedures.

Corresponding Author: A.J. Afolayan, Department of Botany, University of Fort Hare, Alice 5700, South Africa Tel/Fax: +27406022323

Materials and Methods

Plant Material

The berries and leaves of *S. aculeastrum* were collected from trees naturally occurring in the wild at Kayalethu village in the Eastern Cape Province of South Africa (latitudes 30°00'- 34°15'S and longitudes 22°45'-30°15'E). The plant was identified at the Department of Botany, University of Fort Hare and a voucher specimen (Vedic Med 2005/16) was prepared and deposited in the Griffen Herbarium.

Preparation of Extracts

S. aculeastrum fruits were oven dried at 60°C while the leaves were air dried at room temperature. Three equal portions (200 g) of each dried plant material were shaken separately in acetone, methanol and water for 48 h on an orbital shaker. Extracts were filtered using a Buchner funnel and Whatman No. 1 filter paper and each filtrate was concentrated to dryness under reduced pressure at 40°C using a rotary evaporator. The water extracts were freeze dried.

Human Carcinoma Cell Lines and Culture Medium

HT-29 (colonic adenocarcinoma), HeLa (cervical carcinoma) and MCF7 (breast adenocarcinoma) cells were cultured in 10 cm culture dishes in growth medium [antibiotic-free RPMI 1640 medium (Sigma, Germany) containing 10% heat-inactivated fetal bovine serum (Highveld Biological, South Africa), 25 mM HEPES and 2 mM glutamine] in a humidified 5% CO_2 incubator at 37°C. The cancer cell lines were obtained from the American Type Culture Collection (ATCC), USA.

In vitro Cytotoxic Assays

For the determination of cell viability, cells were seeded into 96-well culture plates (Nunclon) at a density of 6000 cells/well in 200 µL aliquots. Cells were allowed to attach for 24 h in a humidified 5% CO_2 incubator at 37°C. Dried fruit and leaf extracts were solubilized in DMSO before further dilution with growth medium. The final concentration of DMSO in the wells never exceeded 0.25%. Cisplatin was used as positive control at concentrations of 10 and 100 µM. Cells were exposed to the extracts or cisplatin for 48 h. Immediately following the 48 h incubation period, cell numbers were determined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay as previously described (Alley *et al*., 1988; Brauns *et al*., 2004). Briefly, cells were incubated with 200 µL MTT (Sigma) (0.5 mg mL in growth medium) for 4 h at 37°C. The formazan product was then dissolved in DMSO and plates were agitated on a shaker for 5 mins, before the absorbance was read at 540 nm on multiwell scanning spectrophotometer (Multiskan MS, Labsystems). The values obtained were used to determine the percentage inhibition of cell growth caused by the extracts (Hagopian *et al*., 1999; Huq *et al*., 2004). Cisplatin was used as a standard control.

Calculations and Statistics

Initial screening for cytotoxicity and log dose-dependent responses were performed in triplicate and quadruplicate, respectively. Results were expressed as percentage growth inhibition of control and treatment values were compared to control values using the Two-sample Students t-test. IC_{50} values for growth inhibition was derived from a nonlinear regression model (curve fit) based on sigmoidal dose response curve (variable) and computed using GraphPadPrism 4 (Graphpad).

Results

Antiproliferative activities of the different extracts of leaves and berries from the *S. aculeastrum* on the growth of three human cancerous cell lines were carried out *in vitro* using tetrazolium assay. Cell proliferation was analyzed at 48 h after cell lines had been cultured with an extracts of 0, 125 and 250 µg mL^{-1} in media while cisplatin was used as positive control. Results of the initial screening

showed that two of the six crude extracts [Fruit Methanol (FM) and Fruit Water (FW)] inhibited the growth of all three tumour cell lines by more than 80% after 48 h exposure ($p<0.001$ for both concentrations tested (Fig. 1). Inhibition by the other four extracts (three extracts of leaves and acetone

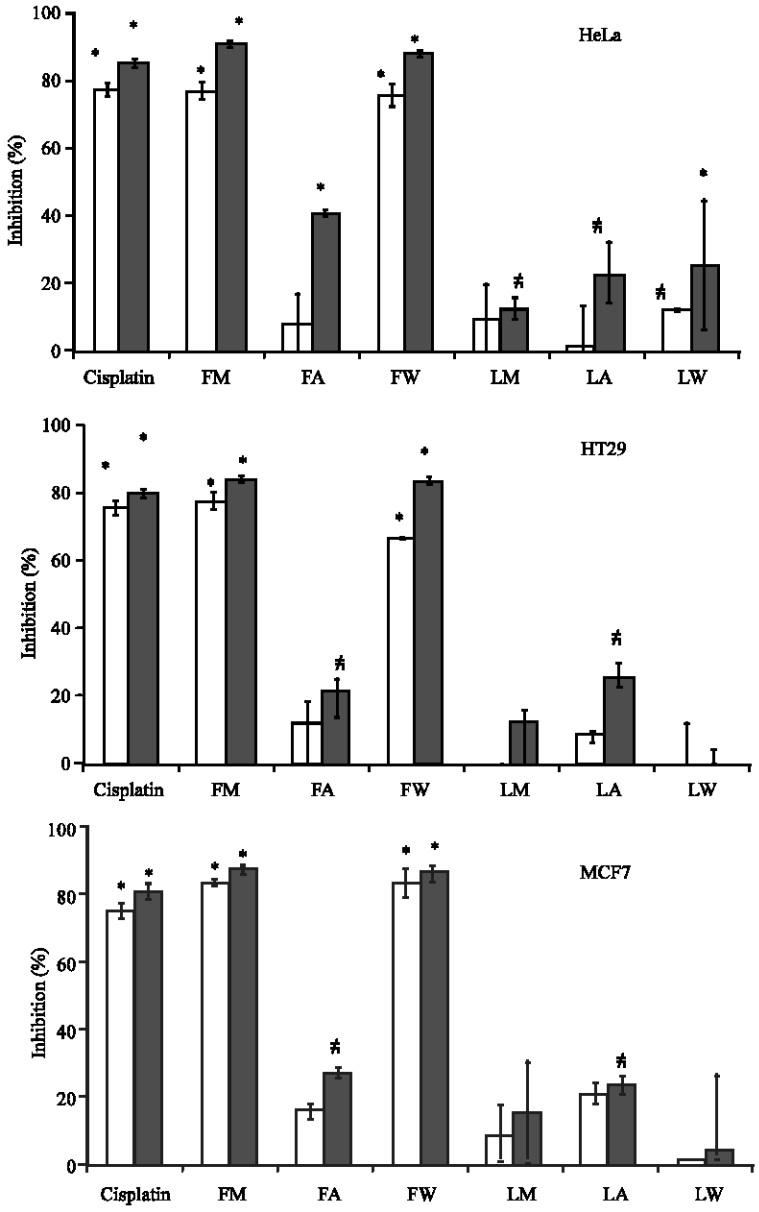

Fig. 1: Initial screening results for six extracts at 125 μg mL^{-1} (clear bars) and 250 μg mL^{-1} (hashed bars) against HeLa, MCF7 and HT29 cells. Cisplatin at 10 μM (clear bars) and 100 μM (hashed bars) was used as a positive control. Error bars represent the standard deviation of triplicate determinations. # $p<0.05$; * $p<0.001$ compared to control (FM = Fruit Methanol; FA = Fruit Acetone; FW = Fruit Water; LM = Leaf Methanol; LA = leaf Acetone; LW = Leaf Water)

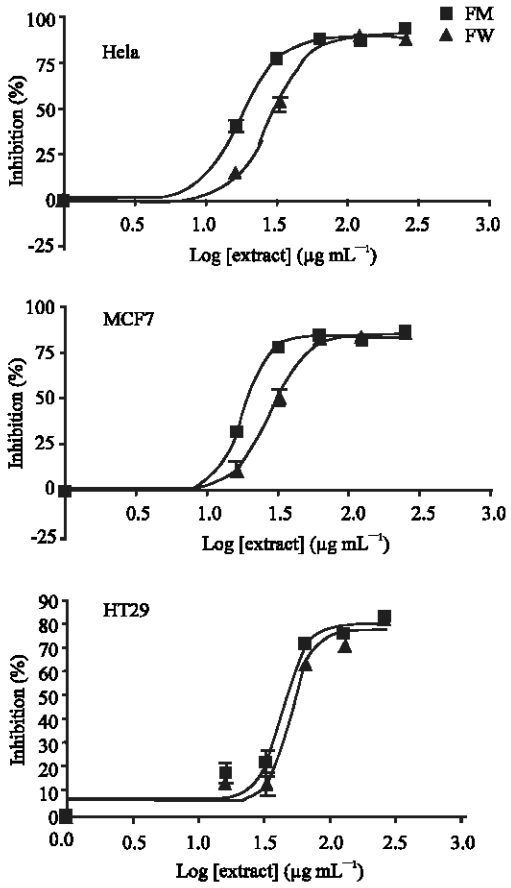

Fig. 2: Log dose-response curves for Fruit Methanol (FM) and Fruit Water (FW) extracts against HeLa, MCF7 and HT29 cells. Error bars represent the standard deviation of quadruplicate determinations

Table 1: IC_{50} values for fruit methanol and fruit water extract for growth inhibition against HeLa, MCF7 and HT29 carcinoma cell lines

	IC50 (µg mL^{-1})	
Cell line	Extract FM	Extract FW
HeLa	17.1	28.4
MCF7	17.8	27.9
HT29	41.9	48.5

extract of berries) were less than 50%, therefore, they were not considered for further testing. The methanol and water extracts of the berries which were further tested against three tumour cell lines at lower concentrations, indicated that IC_{50} values of methanol extract ranged between 17.1 and 41.9 µg mL^{-1} while those of the aqueous extract ranged between 27.9 and 48.5 µg mL^{-1} under experimental conditions (Fig. 2 and Table 1). The methanol extract had a slightly lower IC_{50} than water extract in all the three tumour cell lines tested (Fig. 2 and Table 1). The highest activity was shown on cervical carcinoma (HeLa) and breast adenocarcinoma (MCF7) cell lines and their IC_{50} values for both cell lines were less than 18 µg mL^{-1} (Table 1). This indicates that the extracts were anti cancerous

based on the criterion set by the National Cancer Institute (Geran *et al*., 1972). The leaf extracts had no anticancer activity under the experimental conditions tested.

Discussion

The methanol and water extracts of *S. aculeastrum* was shown to have inhibitory effect on cancer cell lines in the *in vitro* studies. It was effective in the suppression of proliferation of the three cancerous cell lines, HeLa, MCF7 and HT29 in a dose-dependent pattern. A vast variety of naturally occurring substances have been shown to protect against experimental carcinogenesis (Ju *et al*., 2004). Thus, it is becoming increasingly evident that certain phytochemicals, particularly those included in our daily diet, many have important cancer chemopreventive properties (Sanaha *et al*., 1997). In this study, there may be inhibitors and active ingredients in the *S. aculeastrum* extracts, which can induce cytotoxic action against cancer cells and initiate anti proliferative pathway leading to cancer cell death. Traditionally, *S. aculeastrum* is used for the treatment of breast cancer in South Africa. The berries of this plant are boiled in water until they burst into pieces. After filtration, the decoction is administered once a day until signs of relief is observed. This study has confirmed the ethnomedical application of *S. aculeastrum* in the treatment of cancer (Koduru *et al*., 2006c, d). The active compounds in the plant extracts are not yet known. However, plants belonging to the Solanaceae family have been reported to contain complex glycosides and saponins (Tan *et al*., 2005), which may be responsible for the observed activity.

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