

Short Communication

The Anti-Proliferative Properties of Australian Plants with High Antioxidant Capacities Against Cancer Cell Lines

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ABSTRACT: **Introduction:** High levels of antioxidant phytochemicals have been linked to the treatment and prevention of several cancers. Recent reports have identified a number of native Australian fruits as having high antioxidant capacities. Despite this, several of these species are yet to be tested for anticancer activity. **Methods:** Solvent extracts were prepared from high antioxidant native Australian plants and their antioxidant capacities were determined by the DPPH free radical scavenging assay. Antiproliferative activities against CaCo₂ and HeLa cancer cells were determined by an MTS based cell proliferation assay. Toxicity was determined using the *Artemia franciscana* nauplii bioassay. **Results:** The methanolic and aqueous extracts of all plant species displayed high antioxidant contents (equivalent to 16-95 mg of vitamin C per gram of plant material extracted). In contrast, the ethyl acetate extracts for all species had relatively low antioxidant contents (generally below 5 mg of vitamin C equivalents per gram of plant material extracted). The antioxidant contents correlated with the ability of the extracts to inhibit proliferation of CaCo₂ and HeLa cancer cell lines. The high antioxidant methanolic and aqueous extracts of all species were potent inhibitors of cell proliferation, with IC₅₀ values generally below 1000 µg/mL. The aqueous *S. australe* fruit extracts were particularly effective, with IC₅₀ values of 27 and 172 µg/mL against CaCo₂ and HeLa cells respectively. In contrast, the lower antioxidant content ethyl acetate extracts generally did not inhibit cancer cell proliferation. Indeed, exposure of the cancer cells to most of the ethyl acetate extracts induced potent cell proliferation. The methanolic and aqueous *Syzygium* extracts displayed significant toxicity in the *Artemia franciscana* bioassay, with LC₅₀ values below 1000 µg/mL. All other extracts were nontoxic. **Conclusion:** The antiproliferative activity of the high antioxidant plant extracts against HeLa and CaCo₂ cancer cell lines indicates their potential in the treatment and prevention of some cancers.

KEYWORDS: Australian native fruits, *Syzygium*, Davidson's plum, quandong, antioxidant capacity, anticancer activity, antiproliferative activity, functional foods.

INTRODUCTION

Epidemiological studies have shown that a diet rich in antioxidants is associated with a decreased incidence of chronic diseases.¹ High antioxidant levels have also been shown to act as a preventative against the development of degenerative diseases such as cancer,² cardiovascular diseases,³ neural degeneration,⁴ diabetes and obesity.⁵ Phenolic phytochemicals are generally strong antioxidants.⁶

Their primary action involves the protection of cell constituents against oxidative damage through the scavenging of free radicals, thereby averting their deleterious effects on nucleic acids, proteins, and lipids in cells.⁶ Phenolics interact directly with receptors or enzymes involved in signal transduction,⁷ indicating that they play a specific role in human physiology via modulation of the cellular redox state. Common plant phenolic compounds include flavonoids, tannins, anthocyanins, and gallic acid.

The relationship between cellular redox state and cancer progression has been particularly well studied. The induction of cellular oxidative stress has been linked with several types of cancer.^{8,9} Thus it is possible that high antioxidant contents of many fruits may inhibit cancer formation and/or progression. Studies into the antioxi-

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dant/prooxidant effects of extracts from various plant species have demonstrated that the ability of a plant extract to exert antioxidant activity depends on multiple factors. *Aloe vera* antioxidant components for example may function as either an antioxidant or an oxidant, with their action being dependent upon their concentration.¹⁰ The *Aloe vera* anthraquinone aloe emodin exerts antioxidant behaviour at lower concentrations, yet acts as a prooxidant at high concentrations. In contrast, a different *Aloe vera* anthraquinone (aloin) has an antioxidant effect at higher concentrations, yet a prooxidant effect at low concentrations. Thus, *Aloe vera* extracts and components may act as either antioxidants or as oxidants, dependent on differing levels of the various constituents, and on their ratios. Thus, although many plant species have very high antioxidant contents, it is possible that the individual components may act as either antioxidants or as oxidants and thus may also be effective in the treatment of cancer, as well as in its prevention at different concentrations.

Similar prooxidant effects have been reported for other antioxidant phytochemicals including flavonoids¹¹ and tannins.¹² Previous studies have also shown that the presence of transition metal ions such as copper or iron in an extract can further enhance the conversion of the antioxidant to the prooxidant state.^{13,14} The prooxidant/antioxidant effect of plant extracts is due to a balance between the free radical scavenging activities and reducing power of their phytochemical components. This can be explained using the antioxidant vitamin ascorbic acid as an example. Although ascorbic acid has well characterised antioxidant bioactivities, it is also known to act as a prooxidant at high concentrations.¹⁵ This is due to the greater reducing power of ascorbic acid compared to its free radical scavenging activity. In the presence of transition metal ions, ascorbic acid will function as a reducing agent, reducing the metal ions. In this process, it is converted to a prooxidant. Therefore, high dietary intake of ascorbic acid (or other antioxidants) in individuals with high iron levels (eg. premature infants) may result in unexpected health effects due to the induction of oxidative damage to susceptible biomolecules.^{16–18}

Recent studies have documented the exceptionally high antioxidant content of the fruits of several Australian plant species.^{19,20} In particular, these studies reported the fruit of *Syzygium leubmanni*, *Syzygium australe* and *Davidson's pruriens* to have similar antioxidant capacities to blue berries (which are themselves considered to have a high antioxidant capacity). It has previously been postulated that the high antioxidant contents of some Australian native fruits may provide them with therapeutic

effects.^{10,21,22} *Terminalia ferdinandiana* (Kakadu plum) has been reported to have antibacterial activity.²³ Similarly, *Tasmannia lanceolata* extracts have been shown to have potent antibacterial activity.²⁴ Recent studies have also suggested that *T. lanceolata* extracts may also have proapoptotic effects and thus may be cytotoxic to cancer cells.²⁵ Several studies have also reported on the antimicrobial properties of *S. leubmannii* and *S. Australe* fruit²⁶ and leaf extracts.^{27–29} Despite these recent studies, reports of the anticancer activities of many of the high antioxidant Australian plants are lacking. This study examines the antiproliferative activity of *S. leubmannii* (riberry), *S. australe* (brush cherry), *D. pruriens* (Davidson's plum) and *Elaeocarpus angustifolius* (blue quandong) against HeLa and CaCo₂ cancer cell lines.

MATERIALS AND METHODS

Plant source and extraction

D. pruriens, *E. angustifolius*, *S. leubmannii* and *S. australe* fruit and leaves were obtained from the Mt Cootha Botanical Gardens, Brisbane, Australia. All plant materials were identified by Philip Cameron, chief botanist, Mt Cootha Botanical Gardens. Voucher specimens are stored in the School of Natural Sciences, Griffith University, Australia. All plant materials were thoroughly dried in a Sunbeam food dehydrator and the dried plant materials were subsequently stored at -30°C. Prior to use, the plant materials were thawed and freshly ground to a coarse powder. Individual 1 g quantities of the ground leaves and fruit were weighed into individual tubes and 50 ml of methanol, deionised water or ethyl acetate were added. All solvents were obtained from Ajax and were AR grade. The ground fruit and leaves were individually extracted in each solvent for 24 hours at 4°C with gentle shaking. The extracts were filtered through filter paper (Whatman No. 54) under vacuum, followed by drying by rotary evaporation in an Eppendorf concentrator 5301. The resultant dry extract was weighed and redissolved in 10 ml deionised water.

Qualitative phytochemical studies

Phytochemical analysis of the extracts for the presence of saponins, phenolic compounds, flavonoids, polysteroids, triterpenoids, cardiac glycosides, anthraquinones, tannins and alkaloids was conducted by previously described assays.^{30–33}

Antioxidant capacity

The antioxidant capacity of each sample was assessed using the DPPH free radical scavenging method³⁴ with

modifications. Briefly, DPPH solution was prepared fresh each day as a 400 μM solution by dissolving DPPH (Sigma) in AR grade methanol (Ajax, Australia). The initial absorbance of the DPPH solution was measured at 515 nm using a Molecular Devices, Spectra Max M3 plate reader and did not change significantly throughout the assay period. A 2 ml aliquot of each extract was evaporated and the residue resuspended in 2 ml of methanol. Each extract was added to a 96-well plate in amounts of 5, 10, 25, 50, 75 μl in triplicate. Methanol was added to each well to give a volume of 225 μl . A volume of 75 μl of the fresh DPPH solution was added to each well for a total reaction volume of 300 μl . A blank of each extract concentration, methanol solvent, and DPPH was also performed in triplicate. Ascorbic acid was prepared fresh and examined across the range 0–25 μg per well as a reference and the absorbances were recorded at 515. All tests were performed in triplicate and triplicate controls were included on each plate. The antioxidant capacity based on DPPH free radical scavenging ability was determined for each extract and expressed as μg ascorbic acid equivalents per gram of original plant material extracted.

Screen for anticancer bioactivity

Cancer cell lines

The CaCo_2 and HeLa carcinoma cell lines used in this study were obtained from American Type Culture Collection (Rockville, USA). The cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Life Technologies), supplemented with 20 mM HEPES, 10 mM sodium bicarbonate, 50 $\mu\text{g}/\text{ml}$ streptomycin, 50 IU/ml penicillin, 2 mM glutamine and 10% foetal calf serum (Life Technologies). The cells were maintained as mono layers in 75 ml flasks at 37°C, 5% CO_2 in a humidified atmosphere until approximately 80% confluent.

Evaluation of cancer cell antiproliferative activity

Anti proliferation of the extracts was assessed as previously described.³⁵ Briefly, 1 ml of trypsin (Sigma) was added to the culture flasks and incubated at 37°C, 5% CO_2 for 15 min to dislodge the cancer cells. The cell suspensions were then transferred to a 10 ml centrifuge tube and sedimented by centrifugation. The supernatant was discarded and the cells were resuspended in 9 ml of fresh media. Aliquots of the resuspended cells (70 μl , containing approximately 5000 cells) were added to the wells of a 96 well plate. A volume of 30 μl of the test extracts or cell media (for the negative control) was added to individual wells and the plates were incubated at 37°C, 5% CO_2 for 12 hours in a humidified atmosphere. A volume of 20 μl

of Cell Titre 96 Aqueous One solution (Promega) was subsequently added to each well and the plates were incubated for a further 3 hours. Absorbances were recorded at 490 nm using a Molecular Devices, Spectra Max M3 plate reader. All tests were performed in at least triplicate and triplicate controls were included on each plate. The antiproliferative activity of each test was calculated as a percentage of the negative control using the following formula:

$$\text{Proliferation (\% untreated control)} = (A_{ct}/A_{cc}) \times 100$$

A_{ct} is the corrected absorbance for the test extract (calculated by subtracting the absorbance of the test extract in media without cells from the extract cell test combination) and A_{cc} is the corrected untreated control (calculated by subtracting the absorbance of the untreated control in media without cells from the untreated cell media combination).

Toxicity screening

Reference toxin for toxicity screening

Potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) (AR grade, Chem-Supply, Australia) was prepared as a 1.6 mg/ml solution in distilled water and was serially diluted in artificial seawater for use in the *Artemia franciscana* nauplii bioassay.

Artemia franciscana nauplii toxicity screening

Toxicity was tested using a modified *Artemia franciscana* nauplii lethality assay.^{36–39} Briefly, 400 μl of seawater containing approximately 43 (mean 43.2, $n = 155$, SD 14.5) *A. franciscana* nauplii were added to wells of a 48 well plate and immediately used for bioassay. A volume of 400 μl of diluted plant extracts or the reference toxin were transferred to the wells and incubated at $25 \pm 1^\circ\text{C}$ under artificial light (1000 Lux). A negative control (400 μl seawater) was run in triplicate for each plate. All treatments were performed in at least triplicate. The wells were checked at regular intervals and the number of dead counted. The nauplii were considered dead if no movement of the appendages was observed within 10 seconds. After 24h all nauplii were sacrificed and counted to determine the total % mortality per well. The LC_{50} with 95% confidence limits for each treatment was calculated using probit analysis.

Statistical analysis

Data are expressed as the mean \pm SEM of at least three independent experiments. One way ANOVA was used to calculate statistical significance between control and treated groups with a P value < 0.01 considered to be statistically significant.

RESULTS

Liquid extraction yields and qualitative phytochemical screening

Extraction of 1 g of the various dried plant materials with the solvents yielded dried plant extracts ranging from 2.5 mg (*E. angustifolius* fruit ethyl acetate extract) to 560 mg (*S. leubmannii* fruit methanolic extract) (Table 1). Methanolic extracts generally gave relatively high yields of dried extracted material whilst the aqueous extracts had moderate yields for most species. Ethyl acetate extracted lower masses for both the fruit and leaves. The dried extracts were re-suspended in 10 ml of deionised water resulting in the extract concentrations shown in Table 1.

Qualitative phytochemical studies (Table 1) showed that methanol and water extracted the widest range of phytochemicals. Both showed moderate to high levels of phenolics (both water soluble and insoluble phenolics) and flavonoids, as well as low levels of saponins, triterpenoids and tannins. The ethyl acetate extracts generally only had moderate levels of phenolics and low levels of flavonoids, with no other class of phytochemical evident. Alkaloids, cardiac glycosides and anthraquinones were not detected in any of the extracts.

Antioxidant content

Antioxidant capacity (expressed as ascorbic acid equivalence) for the fruit and leaf extracts are shown in Table 1. The antioxidant capacity ranged from a low of 1.5 mg

ascorbic acid equivalence per gram of dried plant material extracted (*S. leubmannii* and *D. pruriens* ethyl acetate extracts) to a high of 95 mg ascorbic acid equivalence per gram of dried plant material extracted (*S. leubmannii* fruit methanolic extract). The methanol extracts generally had higher antioxidant capacities than the water or ethyl acetate extracts, with the exception of the *S. Leubmannii* leaf extracts where the aqueous extract displayed the highest antioxidant capacity.

Antiproliferative activity

Aliquots of each extract were tested for the ability to block cell proliferation of HeLa (Figure 1) and CaCo₂ (Figure 2) cell lines. Sixteen of the 21 plant extracts tested displayed significant ($p < 0.01$) antiproliferative effects against HeLa cells. A further extract (*S. leubmannii* fruit ethyl acetate extract) showed minor inhibition of HeLa cell proliferation, although this inhibition was not significant (5.6% inhibition compared to the negative control cell proliferation). All of the methanolic and aqueous extracts strongly inhibited HeLa cell growth. In contrast, only 2 of the ethyl acetate extracts (*S. australe* and *D. pruriens* fruit extracts) significantly inhibited HeLa cell proliferation. Interestingly, 4 of the ethyl acetate extracts induced significant HeLa cell proliferation (*S. leubmannii* leaf, *S. australe* leaf, *D. pruriens* leaf and *E. angustifolius* fruit). Inhibition of proliferation was dose dependent, with the level of inhibitory activity decreasing at lower concentrations (Table 2). The dose dependent proliferative activity of the ethyl acetate extracts were not evaluated in this study.

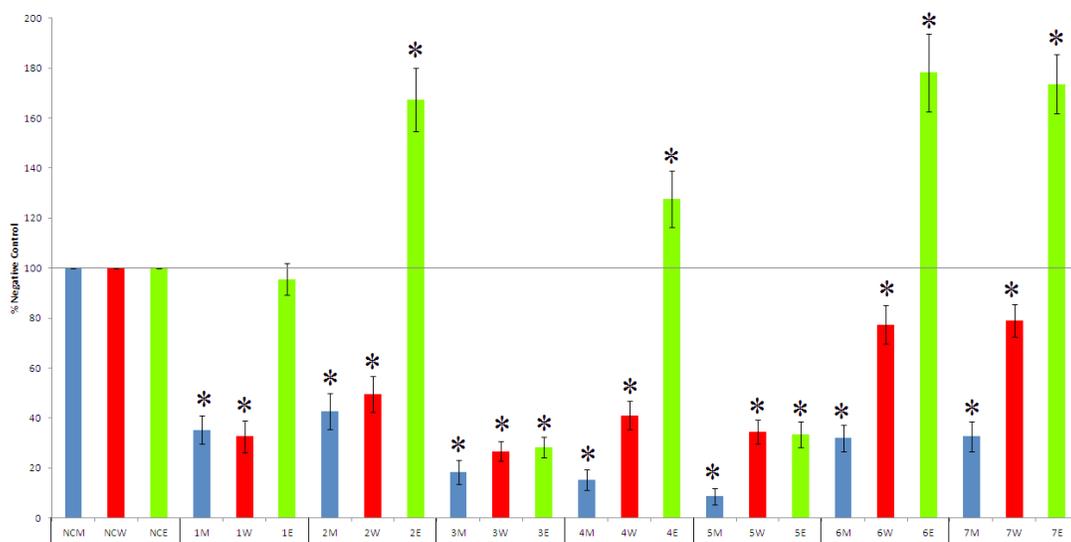


Figure 1. Anti-proliferative activity of plant extracts and untreated controls against HeLa cancer cell lines measured as percentages of the untreated control cells. 1 = *S. leubmannii* fruit; 2 = *S. leubmannii* leaf; 3 = *S. australe* fruit; 4 = *S. australe* leaf; 5 = *D. pruriens* fruit; 6 = *D. pruriens* leaf; 7 = *E. angustifolium* fruit. M = methanolic extract; W = water extract; E = ethyl acetate extract. Results are expressed as mean percentages \pm SEM of at least triplicate determinations. * indicates results that are significantly different to the untreated control ($p < 0.01$).

Table 1: The mass of dried extracted material, the concentration after resuspension in deionised water, qualitative phytochemical screenings and antioxidant capacities (mg equivalence of ascorbic acid/g dried plant material) of plant extracts.

	S. leuhmannii fruit			S. leuhmannii leaf			S. australae fruit			S. australae leaf			D. pruriens fruit			D. pruriens leaf			E. angustifolius fruit			
	W	E	M	W	E	M	W	E	M	W	E	M	W	E	M	W	E	M	W	E	M	
Mass of dried extract (mg)	560	120	130	190	19	88	62	36	240	110	360	180	110	530	220	290	230	36	44	490	140	25
Concentration of resuspended extract (mg/ml)	56	12	13	19	36	9	6	36	24	11	36	18	11	53	22	29	23	4	4	49	14	3
Phenolics	+++	+++	++	+++	+++	+++	++	+++	+++	++	+++	+++	++	+++	+++	++	++	++	++	+++	+++	++
	+++	+++	+++	+++	+++	-	+++	+++	-	+++	+++	-	+++	+++	+++	++	++	-	++	+++	+++	-
	+++	++	+++	+++	+++	++	+++	+++	++	++	+++	++	++	+++	+	++	+	+	++	+++	++	++
Cardiac glycosides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saponins	+	+	-	+	+	+	-	-	+	-	+	+	-	+	-	+	+	+	-	+	-	+
Triterpenes	+	+	-	+	+	+	-	+	-	-	+	+	-	+	+	-	+	+	-	+	+	-
Polysteroids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Meyer test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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Table 1: The mass of dried extracted material, the concentration after resuspension in deionised water, qualitative phytochemical screenings and antioxidant capacities (mg equivalence of ascorbic acid/g dried plant material) of plant extracts.

	S. leuhmannii fruit			S. leuhmannii leaf			S. australae fruit			S. australae leaf			D. pruriens fruit			D. pruriens leaf			E. angustifolius fruit			
	W	E	M	W	E	M	W	E	M	W	E	M	W	E	M	W	E	M	W	E	M	
M																						
Tannins	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	-
Free	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Antioxidant content by DPPH reduction (expressed as mg AA equivalence per 1g plant material extracted)	95	59	1.5	43	45	5.5	55	41	9.2	40	25	2.6	23	16	1.5	35	39	5.5	20.8	22.6	1.2	

+++ indicates a large response; ++ indicates a moderate response; + indicates a minor response; - indicates no response in the assay. AA = ascorbic acid

The extracts were similarly effective at inhibiting CaCo₂ cancer cell proliferation. Fifteen of the 21 plant extracts tested displayed significant ($p < 0.01$) antiproliferative effects against the CaCo₂ cell line. Indeed, the *S. australe* fruit aqueous extract (Figure 2, 3W) completely blocked CaCo₂ cell proliferation (100% inhibition). As with HeLa cell proliferation, all methanolic and aqueous extracts were effective inhibitors of CaCo₂ cellular proliferation. In

contrast, of the ethyl acetate extracts, only the *D. pruriens* extract inhibited CaCo₂ cell proliferation. All of the other ethyl acetate extracts induced significant CaCo₂ cell proliferation. Inhibition of proliferation was dose dependent, with the level of inhibitory activity decreasing at lower concentrations (Table 2). The dose dependent proliferative activity of the ethyl acetate extracts were not evaluated in this study.

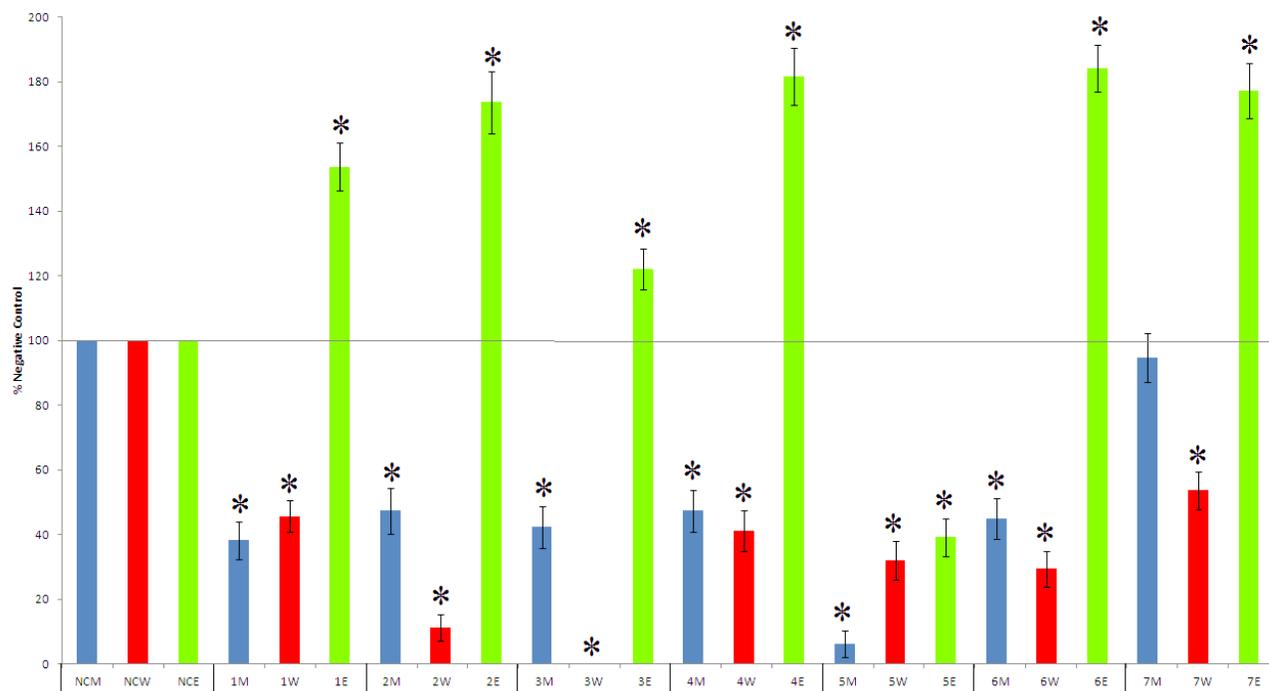


Figure 2. Anti-proliferative activity of plant extracts and untreated controls against CaCo₂ cancer cell lines measured as percentages of the untreated control cells. 1 = *S. leubmannii* fruit; 2 = *S. leubmannii* leaf; 3 = *S. australe* fruit; 4 = *S. australe* leaf; 5 = *D. pruriens* fruit; 6 = *D. pruriens* leaf; 7 = *E. angustifolium* fruit. M = methanolic extract; W = water extract; E = ethyl acetate extract. Results are expressed as mean percentages \pm SEM of at least triplicate determinations. * indicates results that are significantly different to the untreated control ($p < 0.01$).

The antiproliferative efficacy was further quantified by determining the IC₅₀ values for each extract which inhibited cell proliferation against each cell line (Table 2). Most of the extracts were effective at inhibiting cancer cell proliferation at low to moderate concentrations, with IC₅₀ values against both cell lines generally less than 1000 μ g/ml. The aqueous extracts were particularly effective with IC₅₀ values as low as 27 μ g/ml for the *S. australe* fruit aqueous extract against the CaCo₂ cell line. Several other extracts were similarly potent antiproliferative agents, although the IC₅₀ values of most extracts was at least an order of magnitude higher.

Quantification of toxicity

All extracts were initially screened at 2000 μ g/ml in the assay (Figure 3). For comparison, the reference toxin potassium dichromate (1000 μ g/ml) was also tested in the bioassay. (Figure 3) shows the % mortality induced by each extract and by the control toxin following 24 hours exposure. The potassium dichromate reference toxin was rapid in its onset of mortality, inducing mortality within the first 3 hours of exposure and 100% mortality was evident following 4-5 hours (unpublished results). Similarly, the *S. Leubmannii* (fruit and leaf), *S. australe* (fruit and leaf),

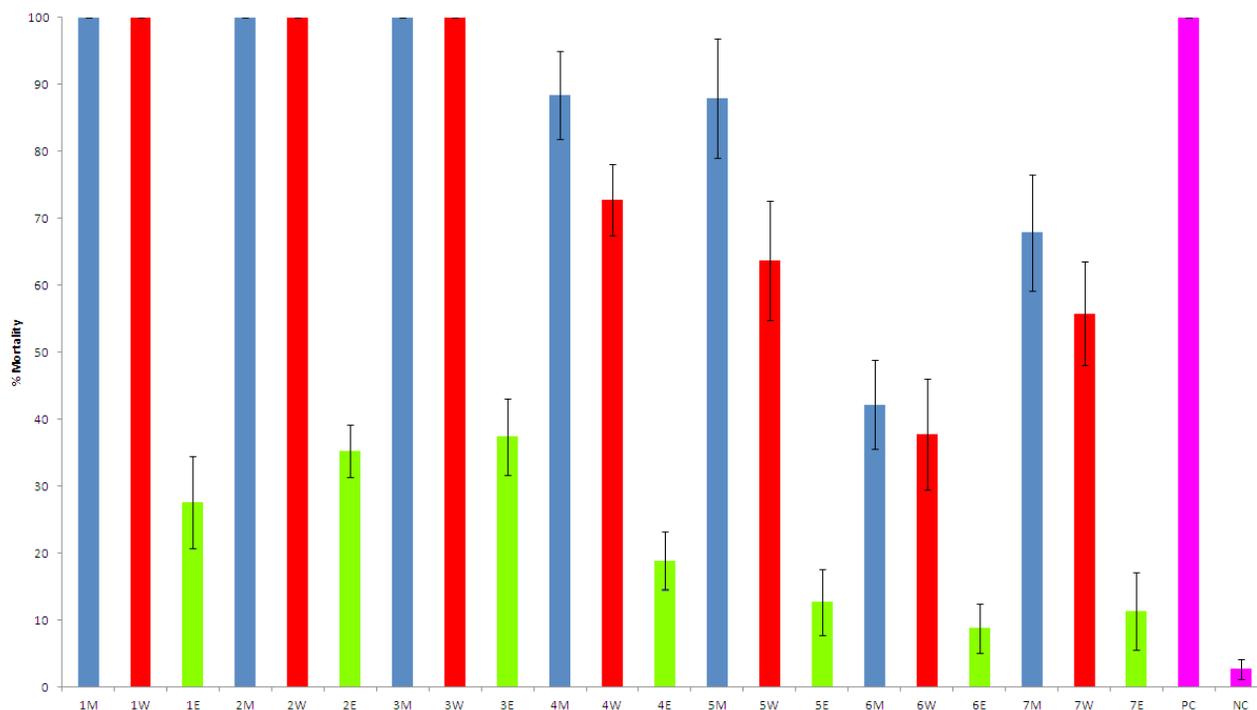


Figure 3. The lethality of the extracts (2000 $\mu\text{g}/\text{mL}$) and the potassium dichromate control (1000 $\mu\text{g}/\text{mL}$) towards *Artemia nauplii* following 24 hours exposure. 1 = *S. leubmannii* fruit; 2 = *S. leubmannii* leaf; 3 = *S. australe* fruit; 4 = *S. australe* leaf; 5 = *D. pruriens* fruit; 6 = *D. pruriens* leaf; 7 = *E. angustifolium* fruit; M = methanolic extract; W = water extract; E = ethyl acetate extract. PC = positive control (1000 $\mu\text{g}/\text{mL}$ potassium dichromate); NC = negative control. All tests were performed in at least triplicate and the results are expressed as mean \pm SEM.

D. pruriens (fruit) and *E. angustifolium* (fruit) methanolic and aqueous extracts displayed $> 50\%$ mortality rates at 24h.

To further quantify the effect of toxin concentration on the induction of mortality, the extracts were serially diluted in artificial sea water to test across a range of concentrations in the *Artemia nauplii* bioassay. (Table 2) shows the LC50 values of the extracts towards *A. franciscana*. No LC50 values are reported for the *D. pruriens* leaf extracts or for any ethyl acetate extract as less than 50% mortality was seen for all concentrations tested. All *D. pruriens* and *E. angustifolius* extracts displayed low toxicity (generally much greater than 1000 $\mu\text{g}/\text{mL}$) following 24 h exposure. Similarly, the *S. australe* fruit extracts also had high LC50 values. Extracts with an LC50 of greater than 1000 $\mu\text{g}/\text{mL}$ towards *Artemia nauplii* have been defined as being nontoxic.⁴⁰ In contrast, the *S. leubmannii* fruit and leaf and the *S. australe* leaf methanolic and aqueous extracts all displayed IC50 values which indicate moderate to high toxicity. As *S. leubmannii* and *S. australe* have previously been shown to have high ascorbic acid contents²⁰ which may significantly decrease the assay pH, this may account for these apparent toxicities.⁴⁰

DISCUSSION

Maintenance of the cellular redox state is required for the normal functioning of living systems. This redox maintenance involves a regulated balance between oxidants and antioxidants. Cells use a variety of different antioxidant mechanisms to help maintain this redox state.²¹ These cellular defences include enzymatic antioxidants (superoxide dismutase, catalase, thioredoxin, thioredoxin reductase, glutathione peroxidase and glutathione peroxidase) and nonenzymatic antioxidants (glutathione, vitamins A, C and E). Deregulation of the redox balance has been implicated in numerous diseases, including atherosclerosis, diabetes, cirrhosis,¹⁶ autoimmune disease and chronic inflammation,⁴¹ neurodegeneration¹⁶ and cancer, (particularly in tumour initiation and tumour promotion).²¹ An understanding of redox regulation is important in identifying new targets for the development of new drugs for cancer prevention and cancer therapy.

Epidemiological studies have shown that a diet rich in antioxidants is associated with a decreased incidence of chronic diseases.⁴² Individuals with elevated dietary

Table 2: The IC50 values (µg/ml) of HeLa in cervical cancer and CaCo2 colorectal cancer cell lines and the LC50 values (µg/ml) for *Artemia franciscana* exposed to the plant extracts.

	<i>S. leuhmannii</i> fruit		<i>S. leuhmannii</i> leaf		<i>S. australae</i> fruit		<i>S. australae</i> leaf		<i>D. pruriens</i> fruit		<i>D. pruriens</i> leaf		<i>E. angustifolius</i> fruit		Negative Control	Potassium dichromate									
	M	W	E	M	W	E	M	W	E	M	W	E	M	W	E										
HeLa IC50	884	86	DNI	165	128	DNI	134	172	58	187	283	DNI	276	316	305	376	DNI	DNI	859	DNI	DNI	-	-	ND	
CaCo2 IC50	791	124	DNI	387	43	DNI	279	27	DNI	653	325	DNI	169	354	372	212	295	DNI	DNI	DNI	DNI	-	-	ND	
<i>A. franciscana</i> nauplii 24 h LC50	414	478	-	450	813	-	1879	3310	-	294	244	-	6443	2883	-	-	-	-	5418	3762	-	-	-	-	224

Numbers indicate the mean IC50 or LC50 values of triplicate determinations. – indicates that IC50 or LC50 values were not obtained as the % mortality did not exceed 5% at any dose tested. DNI = did not inhibit; ND = not determined.

intakes of non-enzymatic antioxidants such as vitamins A, C and E are less likely to suffer many chronic illnesses and some forms of cancer.^{42,43} High antioxidant levels have also been shown to act as a preventative against the development of neural degeneration.⁴⁴ All of the plant species screened for antiproliferative activity against cancer cells in our study have been previously shown to have high antioxidant contents using Trolox equivalence antioxidant content (TEAC) and photo chemi luminescence (PCL) assays.²⁰ Our study confirmed the high antioxidant contents of these plants using a DPPH free radical scavenging assay.

High antioxidant plant extracts may contain many types of phytochemicals, of which phenolic compounds are prevalent. Phenolic compounds are generally strong antioxidants.⁶ Their primary action involves the protection of cell constituents against oxidative damage through the scavenging of free radicals, thereby averting their deleterious effects on nucleic acids, proteins, and lipids in cells.⁶ Phenolics interact directly with receptors or enzymes involved in signal transduction,⁷ clearly indicating that they play a specific role in human physiology.

Therefore, antioxidants such as those found in Australian high antioxidant fruits may contribute to reducing oxidative damage via non-enzymatic mechanisms and the scavenging of free radicals. However, studies into the medicinal effects of antioxidants have proved confusing, with some studies showing therapeutic effects,^{45,46} whilst other studies indicate that these antioxidants may themselves be toxic.^{38,47} The effects of antioxidants are dose dependent, with low doses behaving as antioxidants, while high doses themselves induce toxicity through the induction of oxidative stress.^{28,48}

Multi cellular organisms require a reducing environment for cell proliferation. This is accomplished when there are high reduced glutathione (GSH) levels and low levels of antioxidant proteins such as super oxide dismutase (SOD), glutathione peroxidase (GPx) and catalase.^{49,50} The redox environment of the cell plays a significant role in cell differentiation as well. In contrast to proliferation, differentiation requires an oxidising environment. Low level of antioxidants induce cell proliferation while high levels inhibit it.⁵¹ Therefore, proliferation favours a reducing environment while differentiation requires an oxidising environment.^{52,53} Thus, ROS could also play a very important physiological role as secondary messengers⁵⁴ and in maintenance of redox balance. Consumption of phenolic antioxidants in fruits may help to reduce the oxidative stress of the cell and the whole organism.

The antiproliferative efficacy of plant extracts of four native Australian species was been examined against two cancer cell lines in vitro; HeLa (cervical) and CaCo-2 (colorectal). The observed antioxidant activity was related to the antiproliferative activity of the cancer cells. All of the extracts with high antioxidant content demonstrated antiproliferative activity against both cancer cell lines. Conversely, extracts which did not display antioxidant activity (all of the ethyl acetate extractions) lacked antiproliferative activity. An interesting trend was noted: the lower antioxidant ethyl acetate extracts not only failed to block cancer cell proliferation, but were observed to stimulate proliferation in 4 of the 7 HeLa cell samples and 6 of the 7 CaCo₂ cell samples.

A previous study on the antioxidant activity of fresh apples reported a similar relationship between antioxidant activity and antiproliferative activity against CaCo2 cells.⁵⁵ It was suggested that the antiproliferative activity was due to the combination of phenolic acids and flavonoids.⁵⁵ This correlates with the results observed in the qualitative phytochemical analysis of this report. Phenolic and flavonoid compounds were observed to be present in high levels all of the extracts that displayed antiproliferative activity, and in much lower levels in the extracts that did not block cell proliferation. Flavonoids are believed to protect cells from disease by shielding lipids, proteins and DNA from oxidative damage.⁵⁶ Previous in vitro bioactivity studies of flavonoids have demonstrate dantiinflammatory, antioxidant, and anticancer activity.⁵⁶ Only the *D. pruriens* fruit extracts displayed antiproliferative activity against both cancer cell lines with all three solvent extracts in our study. Individual extract components responsible for the antiproliferative activity of the plant extracts were not identified in the current study.

Whilst the use of high levels of antioxidants may be an attractive option in the prevention and treatment some cancers, oxidative stress is not the sole factor in the development and progression of cancer. Genetic factors, dietary habits and environmental factors may all contribute to cancer development. Further more, even in oxidative stress induced cancers, dietary antioxidants are of limited value as it is difficult to supply enough of the beneficial antioxidants directly in the food to function effectively as chemo-protectants and/or chemo-therapeutics. Instead, high antioxidant extracts may be required to obtain a high enough dosage to be effective. Despite this, numerous studies have reported the beneficial effects of using high levels of phytochemical antioxidants in cancer prevention and treatment and the high antioxidant

Australian native fruits examined in this report may also prove to be useful anticancer agents.

The findings reported here also demonstrate that the majority of the fruit and leaf extracts were nontoxic towards *Artemia franciscana* nauplii. Of the extracts tested, only the ethanolic and aqueous extracts of *Syzygium leuhmannii* leaf and fruit and of *Syzygium australe* fruit displayed LC50 values less than 1000 µg/mL. Fruits of both of these species have previously been reported to have high vitamin C contents.²⁰ As *Artemia* nauplii are susceptible to pH changes³⁸ and to vitamin C,⁴⁰ it is possible the mortality induced by these extracts is due to their high vitamin C contents. All other extracts examined in this study were nontoxic, although further studies using human cell lines are required to verify the safety of these extracts for therapeutic use.

CONCLUSION

The results of this study demonstrate the potential of high antioxidant Australian plants to block proliferation of some cancer cells. The low toxicity of most of the extracts further indicates their potential in the treatment and prevention of some cancers. Purification and identification of the bio active components is needed to examine the mechanisms of action of these agents.

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