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Original Article

Extracts Prepared from Selected High Antioxidant Australian Plants Inhibit Proliferation of HeLa and Caco-2 Carcinoma Cells

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ABSTRACT

Background: High antioxidant foods have been linked to multiple therapeutic properties, including the anticancer and anti-inflammatory properties. Despite this, the anticancer activities of many Australian native plants with high antioxidant capacities are yet to be adequately explored. **Materials and Methods:** The antiproliferative activity of extracts prepared from the fruits and leaves of a selection of high antioxidant Australian plants against human HeLa cervical and Caco-2 colorectal carcinoma cell lines was examined using MTS assays. *Artemia* nauplii lethality assays (ALA) were used to evaluate the therapeutic safety of the extracts. **Results:** Methanolic, aqueous and ethyl acetate extracts prepared from the fruit of *Davidsonia pruriens* were strong inhibitors of proliferation of HeLa and Caco-2 cells, with IC_{50} values 169-376 µg/mL. Although *Elaeocarpus angustifolia, Kunzea pomifera, Podocarpus elatus* and *Acronychia acidula* fruit extracts also inhibited HeLa and Caco-2 proliferation, they were substantially less potent inhibitors of cell proliferation. Additionally, all of the extracts were determined to be non-toxic in the ALA, and the calculated therapeutic indexes highlighted the potential of the extracts for therapeutic use at effective doses. **Conclusion:** The *D. pruriens* extracts were particularly promising inhibitors of HeLa and Caco-2 cell proliferation. Further study is warranted to identify the bioactive components and the mechanism(s) of action.

Keywords: Australian native plants, *Davidsonia pruriens*, Davidson's plum, Anticancer activity, Anti-Proliferative activity, MTS assay, High antioxidant content.

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INTRODUCTION

Several recent studies have evaluated the anti-proliferative activity of high antioxidant plants and reported a link between high antioxidant capacity and antiproliferative activity.[1-3] *Terminalia ferdinandiana* Exell. Fruit extracts were reported to have potent cytotoxic activity against human HT29 colorectal adenocarcinoma and HL60 polymyelocytic leukemia cell lines *in vitro*.[4] That study also reported that *T. ferdinandiana* fruit extracts inhibit the proliferation of non-cancerous human cell lines, but did not significantly affect viability in AGS (human gastric adenocarcinoma) cells. The authors of that study determined that the antiproliferative effects of the *T. ferdinandiana* extract was associated with caspase-7, caspase-9 and poly (ADP-ribose) Polymerase (PARP) activation, indicating that the extracts induced apoptosis via intrinsic pathways. Another study also evaluated the anticancer effects of *T. ferdinandiana* extracts and reported potent antiproliferative activity against human Caco-2 colorectal, HeLa cervical, Jeg3 and JAR choriocarcinomas, as well as the human (MG63) and mouse osteosarcoma cell lines.[5]

Another recent study tested the effects of *Tasmannia lanceolata* (Poir.) A.C.Sm. on the same cell lines and reported similar antiproliferative activities, and determined that the extracts functioned by similar mechanisms.[6] Further studies have also evaluated the antiproliferative properties a variety of high antioxidant Asian,[7-10] Australian.[3,11,12] European[13] and African plants[14] against multiple human carcinoma cell lines. Those studies reported noteworthy anticancer activities for several plant species, and correlated that activity with the antioxidant capacity of the extracts. Despite those earlier studies, the anti-proliferative properties of many other high antioxidant plant species remain to be tested. In particular, Australian native plants have been relatively neglected.

Several studies have identified Australian plant species with high antioxidant capacities and have highlighted their therapeutic potential.[1-3] Those studies reported particularly

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high antioxidant capacities for *Kunzea pomifera* (muntries), *Podocarpus elatus* (Illawarra plum) and *Acronychia acidula* (lemon aspen) fruit.[3,15,16] and linked the antioxidant activity to several therapeutic properties.[1-3,17] Despite the previous studies, many high antioxidant Australian plants are yet to be evaluated for the ability to inhibit carcinoma cell proliferation. Our study screens extracts prepared from the fruit and leaves of selected native Australian plants against human HeLa cervical cancer and Caco-2 colorectal cancer cells. Both of those cell lines have been extensively used to screen for antiproliferative activity in many studies, allowing for direct comparison of efficacies between studies. Furthermore, both cell lines have been well characterised and their susceptibilities and resistances are well documented. Herein, we report the antiproliferative activity of prepared from *D. pruriens* (fruit and leaves), as well as *E. angustifolia, K. pomifera, P. elatus* and *A. acidula* fruit extracts against HeLa and Caco2 cell lines.

MATERIALS AND METHODS

Plant source and extraction

The fruit and leaf materials used in this study were obtained from verified trees in Brisbane, Australia, or were purchased from Go Wild Harvest (Australia) (summarised in Table 1). All plant materials were extensively dehydrated in a Sunbeam food dehydrator until completely desiccated (as a determined by no loss of mass following further drying time). Voucher specimens (listed in Table 1) are stored in the School of Environment and Science, Griffith University, Australia. Prior to extraction, the dried plant materials were ground into a coarse powder. Individual 1 g masses of the dried and powdered plant materials were extracted separately for 24 hr at 4°C with 50 mL of either methanol, deionised water or ethyl acetate. All solvents were AR grade and were purchased from Ajax Fine Chemicals, Australia. The extracts were subsequently filtered through Whatman No. 54 filter paper and air dried at room temperature in the shade. The aqueous extracts were lyophilised at -50°C in a freeze dryer. The dried extracts were subsequently weighed to determine the extraction yield, resuspended in 10 mL deionised water (containing 1% DMSO) and stored at 4°C until use.

Qualitative phytochemical studies

Qualitative phytochemical screening of the extracts for the presence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, phenolic compounds, phytoysterols, saponins, triterpenoids, and tannins was conducted by standard assays.[18]

Screen for anticancer bioactivity

Cancer cell lines

Caco-2 colorectal and HeLa cervical carcinoma cells were obtained from American Type Culture Collection (ATCC, Rockville, USA). The cell lines were grown in Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen), supplemented with 20 mM HEPES, 10 mM sodium bicarbonate, 50 µg/mL streptomycin, 50 IU/mL penicillin, 2 mM glutamine and 10% foetal calf serum (Invitrogen, Australia). The cells were grown at 37°C in a humidified 5% CO_2 environment.

Evaluation of cancer cell antiproliferative activity

The antiproliferative activity of all extracts was assessed by standard antiproliferative assays.[5,6] Briefly, 70 µL of each carcinoma cell suspension (containing approximately 5000 cells), was added to the wells of a 96 well plate and 30 µL of the extract dilutions, or cell media (for the negative control) was added. The cells were incubated at 37°C, 5% CO_2 for 12 hr in a humidified atmosphere. Cisplatin (50 µg/mL) was purchased from Sigma, Australia and included on each plate as a positive control. Following the incubation, 20 µL of Cell Titre 96 Aqueous One solution (Promega) was added to each well and the plates were incubated for a further 3 hr under the same conditions. Absorbances were measured at 490 nm using a Molecular Devices, Spectra Max M3 plate reader. All tests and controls were performed three times, each with 3 internal replicates (n=9). Antiproliferative activity was calculated and expressed as a percentage of the negative control using the formula:

$$\text{Proliferation (\% untreated control)} = (\text{Act}/\text{Acc}) \times 100$$

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).

Artemia franciscana nauplii Lethality Assays (ALA)

Toxicity of all extracts was quantified using a modified *Artemia franciscana* nauplii lethality assay.[19] Briefly, 400 µL of the diluted plant extracts were transferred into separate wells of a 48 well plate. Each well contained 400 µL of artificial seawater containing approximately 50 *Artemia* nauplii and incubated at 25±1°C under artificial light (1000 Lux). Potassium dichromate (AR grade, Chem-Supply, Australia) was prepared at 1 mg/mL and included as a reference toxin. A negative control (artificial seawater) was also included on each plate. The percentage of dead nauplii in each well was determined following 24 hr exposure, and LC_{50}'s (with 95% confidence limits) were calculated using probit analysis. All treatments and controls were performed three times, each with internal triplicates (n=9).

Statistical analysis

The data of all experiments is expressed as the Mean±SEM of three independent experiments, each with triplicate internal replicates (n=9). Statistical significance between the control and treated groups was calculated using One Way ANOVA. p values<0.05

were considered to be statistically significant, whilst $p<0.005$ was considered highly significant, unless otherwise stated.

RESULTS

Liquid extraction yields and qualitative phytochemical screening

Analysis of Davidsonia pruriens extracts

The methanolic and aqueous *D. pruriens* fruit extracts both showed moderate to high levels of polyphenolics and flavonoids (Table 2). In contrast, the *D. pruriens* fruit ethyl acetate extract had only moderate levels of polyphenolics and low levels of saponins, whilst all other classes of phytochemicals were either absent or below the detection threshold of these assays. Low levels of saponins were also present in the *D. pruriens* fruit and leaf methanolic and aqueous extracts. In contrast, saponins were not detected in the aqueous fruit extract. Triterpenoids and tannins were also present in low abundances in the *D. pruriens* methanolic and aqueous fruit extracts. Alkaloids, phytosteroids, cardiac glycosides and anthraquinones were not detected in any of the fruit extracts.

Similarly, low to moderate levels of polyphenolics were detected in the *D. pruriens* leaf ethyl acetate extract, although no other classes of phytochemicals were observed in that extract. Low levels of saponins were also present in the *D. pruriens* methanolic and aqueous leaf extracts, with low levels of triterpenoids and tannins also noted. Alkaloids, phytosteroids, cardiac glycosides and anthraquinones were not detected in any *D. pruriens* leaf extracts.

Analysis of *Elaeocarpus angustifolium* extracts

The methanolic and aqueous *E. angustifolius* fruit extracts contained moderate to high levels of polyphenolics, moderate levels of flavonoids, and low levels of triterpenoids (Table 2). In contrast, only a moderate abundances polyphenolics and low levels of flavonoids were detected in the fruit *E. angustifolius* ethyl acetate extract. Additionally, low levels of saponins were present in the methanolic and ethyl acetate extracts, although they were not detected in the aqueous *E. angustifolius* fruit extract. All other phytochemicals classes were absent or below the detection threshold in these assays (Table 2).

Analysis of *K. pomifera* extracts

The *K. pomifera* methanolic and aqueous fruit extracts contained high levels of polyphenolics (Table 2). In contrast, the *K. pomifera* ethyl acetate extract contained moderate levels of polyphenolics. High levels of flavonoids were detected in the methanolic and aqueous *K. pomifera* fruit extracts, with substantially lower levels detected in the ethyl acetate fruit extract. Saponins were present in high levels in the methanolic and aqueous fruit extracts. Triterpenoids and tannins were present in the *K. pomifera* methanolic and aqueous extracts in moderate levels, with moderate levels of tannins also detected in the ethyl acetate extract. Cardiac glycosides, alkaloids and anthraquinones were absent in all *K. pomifera* extracts.

Analysis of *P. elatus* extracts

A high abundance of polyphenolic compounds was detected in both the *P. elatus* fruit methanolic and aqueous extracts (Table 2). In contrast, polyphenolics were present in only low abundance, or were below the detection threshold in the ethyl acetate extract. Flavonoids were also detected in the methanolic, aqueous and ethyl acetate extracts, albeit only in moderate levels. Saponins were detected in high abundance in the methanolic and aqueous *P. elatus* fruit extracts. Moderate levels of triterpenoids and tannins were detected in the methanolic and aqueous fruit extracts, whilst low to moderate levels of anthraquinones were detected in the methanolic and aqueous fruit extracts. Phytosteroids, cardiac glycosides and alkaloids were absent or were below the detection threshold in all *P. elatus* fruit extracts.

Analysis of *A. acidula* extracts

A high abundance of polyphenolics were detected in all of the *A. acidula* fruit extracts (Table 2). Additionally, high levels of flavonoids were detected in the methanolic and aqueous fruit extract, whilst saponins were only detected in high levels in the aqueous *A. acidula* fruit extract. Moderate levels of triterpenoids were also detected in the methanolic, aqueous and ethyl acetate fruit extracts. Interestingly, moderate levels of cardiac glycosides were detected in the *A. acidula* fruit ethyl acetate extract, although they were absent in all other *A. acidula* extracts.

Antiproliferative activity against Caco-2 and HeLa cell lines

Davidsonia pruriens

The *D. pruriens* methanolic, aqueous and ethyl acetate fruit extracts (Figure 1a), as well as the methanolic and aqueous leaf extracts (Figure 1b), significantly inhibited HeLa and Caco-2 cell growth. Indeed, the methanolic *D. pruriens* fruit extract inhibited HeLa proliferation by 92% compared to the negative control. Similarly, the aqueous and ethyl acetate *D. pruriens* fruit extracts inhibited HeLa proliferation by 68% and 80% respectively (Figure 1a). Similarly, methanolic and aqueous *D. pruriens* leaf extracts inhibited HeLa proliferation by 68% and by 24% respectively (Figure 1b). Interestingly, the *D. pruriens* fruit ethyl acetate extract induced a 77% increase in cell proliferation compared to the untreated control.

The *D. pruriens* methanolic and aqueous fruit extracts also significantly inhibited Caco-2 cell proliferation by approximately 92% and 70% respectively (Figure 1a). Similarly, the methanolic and aqueous *D. pruriens* leaf extracts inhibited Caco-2 proliferation by 56% and 72% respectively (Figure 1b). In contrast, the *D. pruriens* leaf ethyl acetate extract did not inhibit HeLa and

Caco-2 cells, and instead induced proliferation of these cell lines by 77% and 82% respectively, compared with the negative control.

Elaeocarpus angustifolium

The methanolic and aqueous *E. angustifolium* fruit extracts significantly inhibited HeLa cell proliferation by 68% and 22% respectively (Figure 2a). In contrast, the *E. angustifolium* ethyl acetate fruit extract induced significant cell proliferation. Indeed, a 17% increase in HeLa cellular proliferation was observed compared to the negative control. Similarly, the *E. angustifolium* methanolic and aqueous fruit extracts also significantly inhibited Caco-2 cell proliferation by 8% and 43% respectively. In contrast, the *E. angustifolium* ethyl acetate fruit extract induced significant cell proliferation, with a 19% increase in cell proliferation observed compared to the negative control.

Kunzea pomifera

The methanolic and aqueous *K. pomifera* fruit extracts also strongly inhibited HeLa cell growth by 49% each, compared to the untreated control (Figure 2b). In contrast, the *K. pomifera* ethyl acetate fruit extract induced HeLa cellular proliferation by 43%. With some notable exceptions, similar trends were noted for the effects of the *K. pomifera* fruit extracts on Caco-2 proliferation. All *K. pomifera* fruit extracts significantly inhibited Caco-2 cell proliferation. Indeed, the methanolic extract inhibited Caco-2 proliferation by 26%, whilst the aqueous fruit extract inhibited 16 % Caco-2 cellular proliferation compared to the negative control. Interestingly, the *K. pomifera* ethyl acetate fruit extract was a more potent inhibitor of Caco-2 proliferation than the methanolic and aqueous fruit extracts, defying the trends noted for the other plants tested. Notably, the *K. pomifera* ethyl acetate fruit extract, which induced the highest HeLa proliferation (43%), was the strongest inhibitor of Caco-2 proliferation (31%).

Podocarpus elatus

The *P. elatus* methanolic and aqueous fruit extracts each inhibited HeLa cell growth by 44% compared to the untreated controls, whilst the ethyl acetate extracts inhibited HeLa cell proliferation by 7% (Figure 2c). Whilst substantially less potent than the corresponding methanolic and aqueous extracts, this inhibition was significantly different to that of the negative control ($p<0.05$). The methanolic, aqueous and ethyl acetate fruit extracts inhibited Caco-2 proliferation by 8%, 23% and 38%.

Acronychia acidula

The *A. acidula* methanolic and ethyl acetate fruit extracts strongly inhibited HeLa cell proliferation by 99% and 84% respectively (Figure 2d). In contrast, no significant difference was observed between the aqueous extract and the negative control for HeLa proliferation. Similar trends were also noted for the effects of the *A. acidula* fruit extracts on Caco-2 cell proliferation. The *A. acidula* methanolic, aqueous and ethyl acetate fruit extracts each significantly inhibiting Caco-2 cell proliferation by 98%, 81% and 80% respectively.

IC_{50} Determination

The antiproliferative activities of the plant extracts were quantified by calculating their IC_{50} values (Table 3). Selection criteria were implemented to grade the effectiveness of plant extracts, with IC_{50} values ≤500 µg/mL classified as potent antiproliferative agents, values >500 µg/mL to ≤1000 µg/mL were classed as good antiproliferative agents, whilst IC_{50} values >1000 µg/mL were categorised as moderate to weak antiproliferative agents. In general, the methanolic and aqueous extracts displayed the most promising antiproliferative agents against both the HeLa and Caco-2 cells, with some notable exceptions. Indeed, all of the

Table 1: Source and voucher numbers for plant specimens used in this study.

Species	Family	Common name	Plant part	Source (supplier or plant location)	Voucher number
D. pruriens	Cunoniaceae	Davidson's plum, ooray	Fruit	Verified tree, Griffith University, Nathan campus, Australia	GU_DpurF2015a
			Leaves	Verified tree, Griffith University, Nathan campus, Australia	GU_DpurL2015a
E. angistifolia	Elaeocarpaceae	Blue marble tree, silver quandong	Fruit	Supplied by GoWild Harvest, Australia	GU_GW_EangF2015a
K. pomifera	Myrtaceae	Muntries, emu apples, native cranberries	Fruit	Supplied by GoWild Harvest, Australia	GU_GW_KPomF2015a
P. elatus	Podocarpaceae	Illawarra plum, brown plum pine,	Fruit	Supplied by GoWild Harvest, Australia	GU_GW_PelF2015a
A. acidula	Rutaceae	Lemon aspen, lemon wood	Fruit	Supplied by GoWild Harvest, Australia	GU_GW_Aacid2015a

Table 2: The mass of dried extracted material, the concentration after resuspension in deionised water, qualitative phytochemical screenings and antioxidant capacities of the plant extracts.

		Yield (mg)	Extract concentration (mg/mL)	Total phenolics	Water soluble phenolics	Water insoluble phenolics	flavonoids	Phytosterols	Saponins	Triterpenoids	Cardiac glycosides	Tannins	Alkaloids	Anthraquinones
D. pruriens	F M	530	53	++	+++	++	+++	-	+	+	-	+	-	-
	F W	220	22	+++	+++	++	++	-	-	+	-	+	-	-
	F E	22	2	++	++	+	-	-	+	-	-	-	-	-
	L M	230	23	++	++	++	+++	-	+	+	-	+	-	-
	L W	36	4	++	++	+	+++	-	+	+	-	+	-	-
	L E	44	4	++	-	+	-	-	-	-	-	-	-	-
E. angustifolia	F M	490	49	+++	++	++	+++	-	+	+	-	-	-	-
	F W	140	14	+++	++	++	++	-	-	+	-	-	-	-
	F E	25	3	++	-	++	+	-	+	-	-	-	-	-
K. pomifera	F M	524	52	+++	+++	-	+++	-	+++	++	-	++	-	-
	F W	350	35	+++	+++	+++	+++	-	+++	++	-	++	-	-
	F E	19	2	+	-	+++	+	-	-	-	-	-	-	-
P. elatus	F M	314	31	+++	+++	+++	++	-	+++	++	-	++	-	+
	F W	195	20	+++	++	+++	++	-	+++	++	-	++	-	+
	F E	3	0.3	+	-	+	++	-	-	-	-	+	-	-
A. acidula	F M	360	36	+++	-	-	+++	-	-	++	+	-	-	-
	F W	162	16	+++	-	-	+++	-	+++	++	+	-	-	-
	F E	67	7	+++	-	+	-	-	-	++	+	-	-	-

+++=large response; ++ moderate response; +=low response; -=absence of phytochemical class in the extract; F-=fruit; L=leaf; M=methanolic extract; W=aqueous extract; E=ethyl acetate extract.

methanolic and aqueous extracts had IC_{50} values substantially <1000 µg/mL. In comparison, the majority of the ethyl acetate extracts had relatively weak antiproliferative agents.

For the antiproliferative extracts, the HeLa anti-proliferative IC_{50} values ranged from 276 µg/mL (*D. pruriens* methanolic fruit extract), to a high of 1560 µg/mL (*A. acidula* ethyl acetate fruit extract). We were unable to determine IC_{50} values for many of the extracts as the inhibition of HeLa proliferation did not exceed 50% at any concentration (designated CND in Table 3). The *D. pruriens* methanolic fruit extract was the most potent antiproliferative agent against HeLa, with an IC_{50} value of 276 µg/mL. All other *D. pruriens* fruit extracts, as well as the corresponding methanolic leaf extract, were similarly potent, with an IC_{50} values in the range of 276-376 µg/mL.

The *D. pruriens* extracts were also the strongest inhibitors of Caco-2 carcinoma proliferation. Indeed, the methanolic, aqueous and ethyl acetate extracts prepared from both *D. pruriens* fruit and leaves each had IC_{50} values substantially <500 µg/mL. For the active extracts, the Caco2 anti-proliferative IC_{50} values ranged from 169 µg/mL (methanolic *D. pruriens* fruit extract) to 1301 µg/mL (ethyl acetate *A. acidula* fruit extract). As noted for the inhibition of HeLa proliferation, we were unable to determine IC_{50} values for many of the extracts against Caco2 as the inhibition induced by those extracts did not exceed 50% at any concentration tested.

Quantification of toxicity

The toxicity of all extracts was initially evaluated at 2000 µg/mL (Figure 3). Potassium dichromate (1000 µg/mL) was also included in the bioassay as a positive control. Several extracts (all ethyl acetate extracts, as well as the methanolic and aqueous *D. pruriens* leaf extracts) failed to induce >50% mortality in the ALA assay at the highest dose tested and were therefore considered to

be non-toxic. In contrast, most extracts displayed >50% mortality at the highest doses tested. All extracts were subsequently screened across a range of dilutions in the ALA bioassay and LC_{50} values were calculated (Table 3). Extracts with LC_{50} values >1000 µg/mL were deemed to be non-toxic,[20] whilst extracts with LC_{50} values <1000 µg/mL were determined to be toxic. As LC_{50} values >1000 µg/mL were calculated for all extracts, all were deemed to be non-toxic in the ALA assay.

DISCUSSION

The majority of clinical anticancer drugs were originally isolated from plants, or are semi-synthetic compounds that use plant natural products as molecular scaffolds. Indeed, approximately three quarters of new antineoplastic drugs approved for clinical use between 1981 and 2002 were developed using plant compound scaffolds.[1] Furthermore, some of the most widely used anticancer drugs are derived from plant compounds, including paclitaxel (derived from taxol isolated from *Taxus brevifolia* Nutt.) and vincristine and vinblastine (isolated from *Catharanthus roseus* (L.) G.Don.). However, new safe and effective anticancer drugs are required to treat highly aggressive cancers, and cancers that have developed resistances to existing clinical chemotherapies. Some highly aggressive cancers, including pancreatic cancer, may require combinations of chemotherapeutics, or high doses of chemotherapeutics used concurrently with radiotherapy to effectively inhibit angiogenesis and metastasis. However, high chemotherapeutic doses and/or the concomitant use of multiple therapy modalities may be highly toxic and can induce multiple serious effects in patients. Additionally, long-term usage of some cancer drugs may induce carcinoma cells to mutate to produce drug resistance mechanisms, thereby reducing the effectiveness of the therapy.[22] Therefore, the development of novel anti-cancer chemotherapies is important to provide a continued pipeline of effective therapies.

Plant extracts with high antioxidant capacities are attractive targets for the development of novel drugs for the treatment and/or prevention of some cancers as oxidative stress is directly involved in cancer etiology and development of many cancers. Thus, modulation of the cellular redox state may be effective against some cancers. Indeed, several recent studies have reported anticancer properties of plants used in traditional medicine and have highlighted their potential for therapeutic usage.[3-5,23-30] All of the plant species examined in our study were selected as all have previously been reported to have high antioxidant capacities,[1-3] and several species have also been reported to have other therapeutic properties.[23-30] It is also important that any new drug should have low toxicity. Notably, each of the plant species examined in our study had previously been reported to be either non-toxic, or have low toxicity.[3,30]

Interestingly, at least one extract of each plant material screened in our study inhibited the proliferation of both HeLa and Caco2. Several species were especially promising inhibitors of HeLa and Caco2 cell proliferation. In particular, the *D. pruriens* fruit and leaf extracts were particularly potent anti-proliferative agents, with IC_{50} values as low as 169 µg/mL (*D. pruriens* fruit extract versus Caco2 cells). A general trend was noted: the degree of inhibition decreased with decreasing polarity across the extracts, even within the same plant species. The methanolic and aqueous extracts had higher extraction yields and were richer in the screened phytochemical classes than the ethyl acetate extracts. Notably, high therapeutic index values were calculated for the methanolic and aqueous *D. pruriens* fruit extracts against both cell lines (8.1-38.1). Thus, doses substantially lower than the toxic dosage are effective against these cells, indicating that the use of relatively low doses of these extracts may be both safe and effective, although this remains to be verified *in vivo*.

Conversely, some of the extracts ethyl acetate extracts not only lacked anti-proliferative activity, but instead induced HeLa and

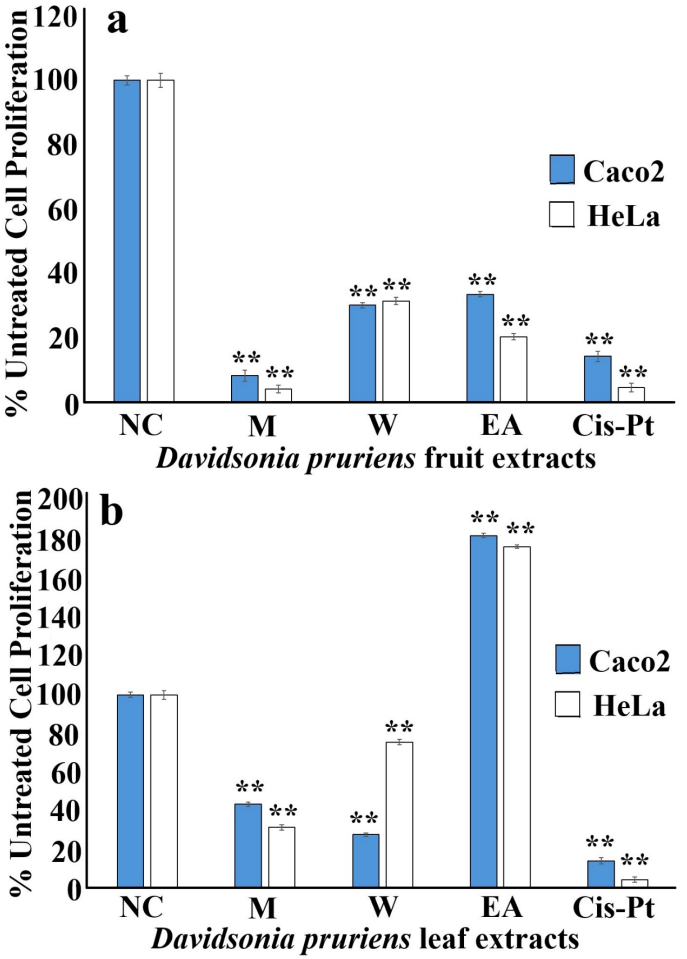

Figure 1: Anti-proliferative activity of the *D. pruriens* (a) fruit and (b) leaf extracts against Caco2 and HeLa cancer cell lines measured as percentages of the untreated control cells. NC=untreated control; M=methanolic extract; W=aqueous extract; E=ethyl acetate extract; Cis-Pt=cisplatin control (50 µg/mL). Results are expressed as mean percentages±SEM of three independent experiments, each with internal triplicates (n=9). * indicates results that are significantly different to the untreated control (p<0.05); ** indicates results that are highly significantly different to the untreated control (p<0.005).

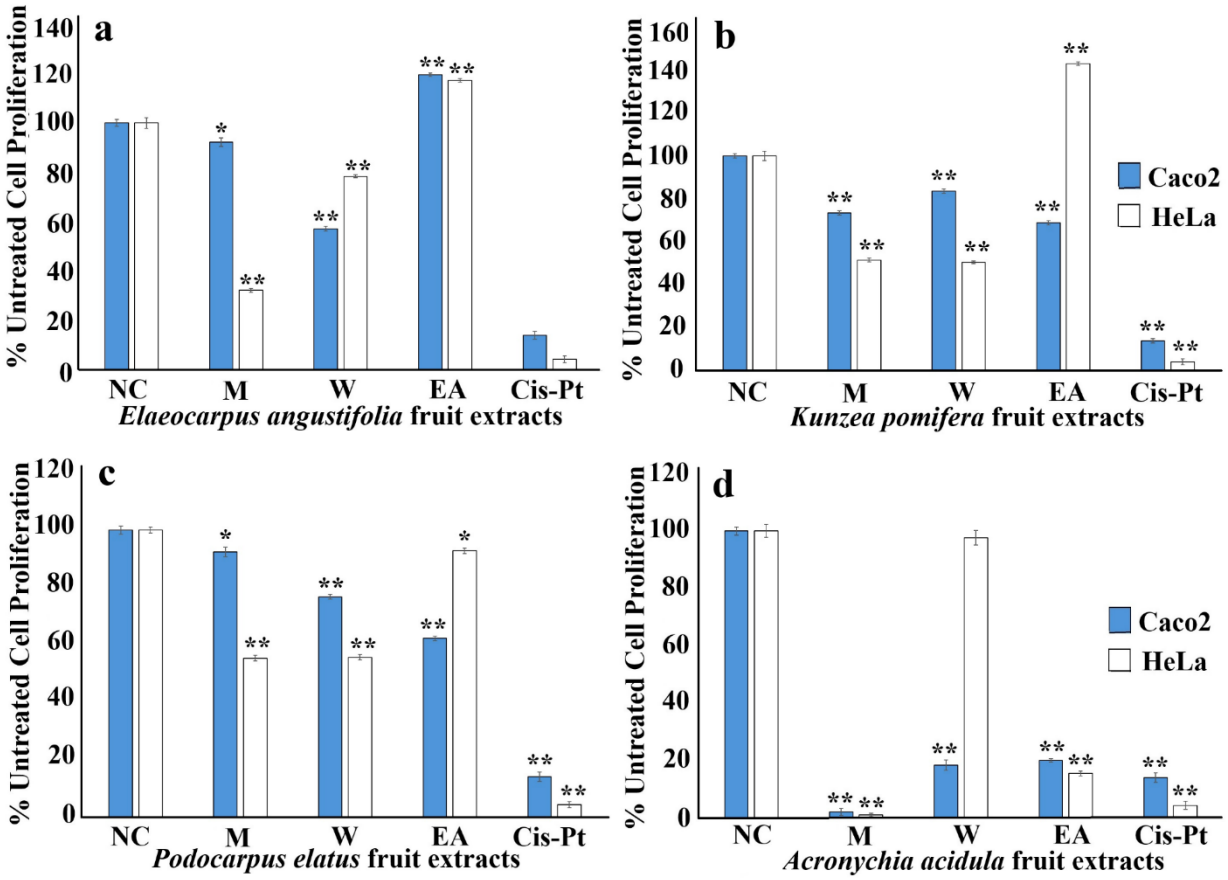

Figure 2: Anti-proliferative activity of the (a) *E. angistifolia*, (b) *K. pomifera*, (c) *P. elatus* and (d) *A. acidula* fruit extracts against Caco2 and HeLa cancer cell lines, measured as percentages of the untreated control cells. NC=untreated control; M=methanolic extract; W=aqueous extract; E=ethyl acetate extract; Cis-Pt=cisplatin control (50 µg/mL). Results are expressed as mean percentages±SEM of three independent experiments, each with internal triplicates (n=9). * indicates results that are significantly different to the untreated control (p<0.05); ** indicates results that are highly significantly different to the untreated control (p<0.005).

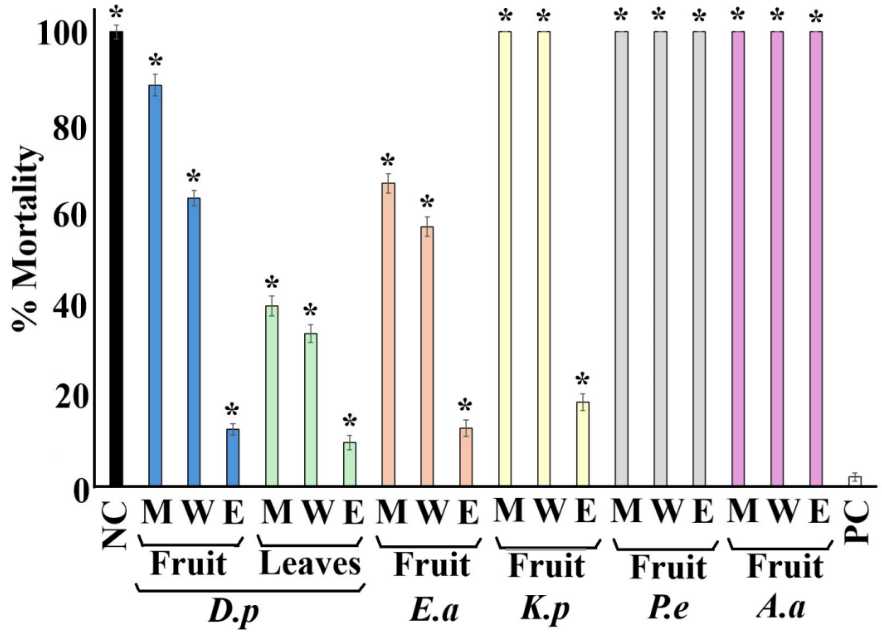

Figure 3: The lethality of the extracts (2000 µg/mL) and the potassium dichromate control (1000 µg/mL) towards Artemia nauplii following 24 hr exposure. *D.p=D. pruriens; E.a=E. angistifolia; K.p=K. pomifera; P.e=P. elatus; A.a=A. acidula;* NC=negative control; M=methanolic extract; W=aqueous extract; E=ethyl acetate; PC=positive control (1000 µg/mL potassium dichromate). Results are expressed as Mean±SEM of three independent experiments, each with internal triplicates (n=9). * indicates results that are significantly different to the negative control (p<0.01).

Table 3: IC_{50} values of the plant extracts following 24 hr exposure against Caco-2 and HeLa carcinoma cell lines (µg/mL), as well as LC_{50} values in *Artemia* lethality assays and therapeutic Index (TI values).

Plant Species and Part		Caco-2			HeLa			LC_{50}			TI against Caco-2			TI against HeLa		
		M	W	E	M	W	E	M	W	E	M	W	E	M	W	E
D. pruriens	Fruit	169	354	372	276	316	305	6443	2883	CND	38.1	8.1	CND	23.3	8.1	CND
	Leaf	212	295	CND	376	CND	CND	CND	CND	CND	CND	CND	CND	CND	CND	CND
E. angustifolia	Fruit	CND	CND	CND	859	CND	CND	5418	3762	CND	CND	CND	CND	6.3	CND	CND
K. pomifera	Fruit	CND	CND	CND	CND	CND	CND	1965	CND	1515	CND	CND	CND	CND	CND	CND
P. elatus	Fruit	CND	CND	CND	CND	CND	CND	1664	1956	1253	CND	CND	CND	CND	CND	CND
A. acidula	Fruit	769	885	1301	480	CND	1560	1500	1872	1609	2	2.1	1.2	3.1	CND	1

All values are expressed in µg/mL and were calculated using Probit analysis (95% confidence interval). M=methanol extract; W=aqueous extract; E=ethyl acetate extract; CND=results that could not be determined as inhibition of proliferation or *Artemia nauplii* mortality did not reach 50% at any concentration tested. Bold and blue results indicate noteworthy IC_{50} values (<1000 µg/mL); Bold and red indicate good TI values (≥4.0).

Caco-2 proliferation. Indeed, the *D. pruriens* leaf ethyl acetate and *E. angustifolium* ethyl acetate fruit extracts, displayed significant proliferative activities. It is possible that the different prolifierative/anti-proliferative effects may relate directly to their antioxidant capacities and substantially more study is required to determine the molecular mechanisms.

CONCLUSION

The results of this study demonstrate the potential of methanol, aqueous and ethyl acetate extracts of the selected high antioxidant Australian plants. The *D. pruriens* extracts a (fruit and leaves) were particularly promising as they were potent inhibitors of HeLa and Caco2 proliferation. High TI values were also calculated for the methanolic extracts, indicating their safety for therapeutic use and further study is warranted on this species. In particular, phytochemical studies to identify the bioactive components and studies to determine the antiproliferative mechanism(s) of these extracts are required to rigorously evaluate their potential as cancer chemotherapeutics.

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ABBREVIATIONS

ALA: Brine-shrimp lethality assay; **DMSO:** Dimethyl sulfoxide; **IC_{50}:** Dose that induces a 50% reduction compared to the untreated control; **LC_{50}:** Dose of sample necessary to have a lethal effect on 50% of test organisms or cells; **RPMI:** Roswell Park Memorial Institute media.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Both authors contributed to this study. J. Shalom and I.E. Cock conceived of and designed the experiments; J. Shalom performed the experiments and contributed to the analysis and interpretation of the data; J. Shalom and I.E. Cock wrote the manuscript.

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