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ORIGINAL ARTICLE

High Performance Liquid Chromatography-mass Spectrometry Analysis of High Antioxidant Australian Fruits with Antiproliferative Activity Against Cancer Cells

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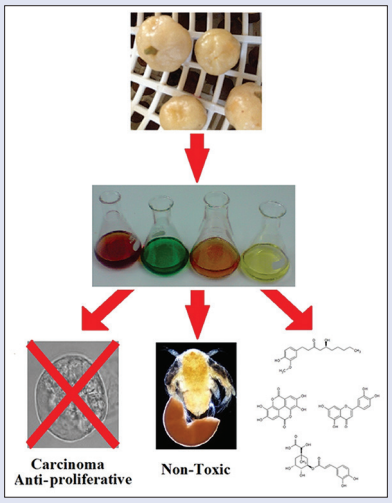
ABSTRACT

Background: High antioxidant capacities have been linked to the treatment and prevention of several cancers. Recent reports have identified several native Australian fruits with high antioxidant capacities. Despite this, several of these species are yet to be tested for anticancer activity. **Materials and Methods:** Solvent extracts prepared from high antioxidant native Australian fruits were analyzed for antioxidant capacity by the di (phenyl)-(2,4,6-trinitrophenyl) iminoazanium free radical scavenging assay. Antiproliferative activities against CaCo2 and HeLa cancer cells were determined by a multicellular tumor spheroid-based cell proliferation assay. Toxicity was determined by *Artemia franciscana* bioassay. **Results:** Methanolic extracts of all plant species displayed high antioxidant contents (equivalent to approximately 7–16 mg of vitamin C per gram of fruit extracted). Most aqueous extracts also contained relatively high antioxidant capacities. In contrast, the ethyl acetate, chloroform, and hexane extracts of most species (except lemon aspen and bush tomato) had lower antioxidant contents (below 1.5 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 extracts of all species were potent inhibitors of cell proliferation. The methanolic lemon aspen extract was particularly effective, with IC50 values of 480 and 769 μg/mL against HeLa and CaCo2 cells, respectively. In contrast, the lower antioxidant ethyl acetate and hexane extracts (except the lemon aspen ethyl acetate extract) generally did not inhibit cancer cell proliferation or inhibited to only a minor degree. Indeed, most of the ethyl acetate and hexane extracts induced potent cell proliferation. The native tamarind ethyl acetate extract displayed low-moderate toxicity in the *A. franciscana* bioassay (LC50 values below 1000 μg/mL). All other extracts were nontoxic. A total of 145 unique mass signals were detected in the lemon aspen methanolic and aqueous extracts by nonbiased high-performance liquid chromatography-mass spectrometry analysis. Of these, 20 compounds were identified as being of particular interest due to their reported antioxidant and/or anticancer activities. **Conclusions:** The lack of toxicity and 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.

Key words: Anticancer activity, bush tomato, desert lime, functional foods, Illawarra plum, lemon aspen, muntries, native tamarind

SUMMARY

- Australian fruit extracts with high antioxidant contents were potent inhibitors of CaCo2 and HeLa carcinoma cell proliferation
- Methanolic lemon aspen extract was particularly potent, with IC50 values of 480 μg/mL (HeLa) and 769 μg/mL (CaCo2)
- High-performance liquid chromatography-mass spectrometry-quadrupole time-of-flight analysis highlighted and putatively identified 20 compounds in the antiproliferative lemon aspen extracts
- In contrast, lower antioxidant content extracts stimulated carcinoma cell proliferation
- All extracts with antiproliferative activity were nontoxic in the Artemia nauplii assay.



Abbreviations used: DPPH: di (phenyl)- (2,4,6-trinitrophenyl) iminoazanium, HPLC: High-performance liquid chromatography, IC50: The concentration required to inhibit by 50%, LC50: The concentration required to achieve 50% mortality, MS: Mass spectrometry.

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INTRODUCTION

Reactive oxygen species (ROS) including superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH•) are products of normal cellular metabolism.[1,2] While they may also be produced for essential biological functions, they are often by-products of incomplete reduction processes.[3] These ROS place the cell under oxidative stress and may damage cellular proteins and lipids, negatively affecting the

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cells chances of survival. Alternatively, ROS may also form/induce the formation of DNA adducts which may promote carcinogenic activity.[1,4,5] Cells respond to oxidative stress via a variety of mechanisms to minimize the effects of ROS. These defenses consist of both enzymatic and nonenzymatic mechanisms. The antioxidant defensive enzymes include superoxide dismutase, catalase, thioredoxin, thioredoxin reductase, glutathione reductase, glutathione peroxidase and glutathione reductase.[3] The nonenzymatic antioxidant defenses include glutathione, as well as vitamins A, C, and E.

High dietary intakes of nonenzymatic antioxidants have been linked with decreased incidence of some chronic diseases, including some cancers.[6] Studies into the antioxidant/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 may function as either an antioxidant or an oxidant, with their action being dependent upon their concentration.[7] The *Aloe vera* anthraquinone aloe emodin exerts antioxidant behavior 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 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 flavonoids[8] and tannins.[9] 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.[10,11] 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-characterized antioxidant bioactivities, it is also known to act as a prooxidant at high concentrations.[12] 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 (e.g., premature infants) may result in unexpected health effects due to the induction of oxidative damage to susceptible biomolecules.[13-15]

Recent studies have documented the exceptionally high antioxidant content of the fruits of several Australian plant species.[16,17] In particular, these studies reported the fruit of *Kunzea pomifera* (muntries) and *Podocarpus elatus* (Illawarra plum) to have similar antioxidant capacities to blueberries (which are themselves considered to have a high antioxidant capacity). Similarly, *Acronychia acidula* (lemon aspen), *Citrus glauca* (desert lime), and *Solanum centrale* (bush tomato) have been reported to have high antioxidant capacities.[18] It has previously been postulated that the high antioxidant contents of some Australian native fruits may provide them with therapeutic effects.[7,16-19] *Terminalia ferdinandiana* (Kakadu plum) has been reported to have antibacterial activity.[20] Similarly, *Tasmannia lanceolata* extracts have been shown to have potent antibacterial activity.[21] Recent studies have also suggested that *T. lanceolata* extracts may also have pro-apoptotic effects and thus, may be cytotoxic to cancer cells.[22] Several studies have also reported on the antimicrobial properties of *Syzygium luehmannii* and *Syzygium australe* fruit[23] and leaf extracts.[24-26] Recently, we reported on the antiproliferative activity of a panel of extracts prepared from selected high antioxidant Australian fruits against two cancer cell lines.[27] Despite these recent studies, reports of the anticancer activities of many of the other high antioxidant Australian plants are lacking. This study examines the antiproliferative activity of *K. pomifera* (muntries), *P. elatus* (Illawarra plum), *Diploglottis australis* (native tamarind), *A. acidula* (lemon aspen), *C. glauca* (desert lime), and *S. centrale* (bush tomato) against HeLa and CaCo2 cancer cell lines.

MATERIALS AND METHODS

Plant source and extraction

All fruits were obtained from taste of Australia online supplier as frozen fruits. 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 fruit were weighed into tubes and 50 ml of methanol, deionized water or ethyl acetate was added. All solvents were obtained from Ajax and were AR grade. The ground fruit was individually extracted in each solvent for 24 h 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 extracts were weighed and redissolved in 10 ml deionized 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.[28-31]

Antioxidant capacity

The antioxidant capacity of each sample was assessed using the di (phenyl)-(2,4,6-trinitrophenyl) iminoazanium (DPPH) free radical scavenging method[27] 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, and 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 CaCo2 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 1640 medium (Life Technologies) and supplemented with 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 10 mM sodium bicarbonate, 50 μg/ml streptomycin, 50 IU/ml penicillin, 2 mM glutamine, and 10% fetal calf serum (Life Technologies). The cells were maintained as monolayers in 75 ml flasks at 37°C, 5% CO_2 in a humidified atmosphere until approximately 80% confluent.

Evaluation of cancer cell antiproliferative activity

Antiproliferation of the extracts was assessed as previously described.[27,32] 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 h in a humidified atmosphere. A volume of 20 μl of CellTiter 96 Aqueous One solution (Promega) was subsequently added to each well and the plates were incubated for a further 3 h. 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:

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 ($K_2Cr_2O_7$) (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 *A. franciscana* nauplii lethality assay.[33-36] Briefly, 400 μl of seawater containing approximately 43 (mean 43.2, $n = 155$, standard deviation 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 was transferred to the wells and incubated at 25 ± 1°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 s. After 24 h, 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.

Nontargeted high performance liquid chromatography-mass spectrometry quadrupole time-of-flight analysis

Chromatographic separations were performed as previously described.[32] Briefly, 2 μL of sample was injected into an Agilent 1290 high-performance liquid chromatography (HPLC) system fitted with a Zorbax Eclipse Plus C18 column (2.1 mm × 100 mm, 1.8 μm particle size). The mobile phases consisted of (A) ultrapure water and (B) 95:5 acetonitrile/water at a flow rate of 0.7 mL/min. Both mobile phases were modified with 0.1% (v/v) glacial acetic acid for MS analysis in positive mode and with 5 mM ammonium acetate for analysis in negative mode. The chromatographic conditions utilized for the study consisted of the first 5 min run isocratically at 5% B, a gradient of B from 5% to 100% was applied from 5 to 30 min, followed by 3 min isocratically at 100%. MS analysis was performed on an Agilent 6530 quadrupole time-of-flight (QTOF) spectrometer fitted with a Jetstream electrospray ionization source in both positive and negative mode.

Data were analyzed using the Masshunter Qualitative analysis software package (Agilent Technologies, Australia). Blanks using each of the solvent extraction systems were analyzed using the Find by Molecular Feature algorithm in the software package to generate a compound list of molecules with abundances >10,000 counts. This was then used as an exclusion list to eliminate background contaminant compounds from the analysis of the extracts. Each extract was then analyzed using the same parameters using the Find by Molecular Feature function to generate a putative list of compounds in the extracts. Compound lists were then screened against three accurate mass databases – a database of known plant compounds of therapeutic importance generated specifically for this study (800 compounds); the Metlin metabolomics database (24,768 compounds); and the Forensic Toxicology Database by Agilent Technologies (7509 compounds). Empirical formula for unidentified compounds was determined using the Find Formula function in the software package.

Statistical analysis

Data are expressed as the mean ± standard error mean of at least three independent experiments. One-way ANOVA was used to calculate statistical significance between control and treated groups, with a $P < 0.01$ considered to be statistically significant.

RESULTS

Liquid extraction yields and qualitative phytochemical screening

Extraction of 1 g of the various dried Australian plant fruits with the solvents yielded dried plant extracts ranging from 3 mg (Illawarra plum fruit ethyl acetate extract) to 524 mg (muntries methanolic extract) [Table 1]. Methanolic extracts generally gave relatively high yields of dried extracted material while the aqueous extracts had moderate to high yields for most species. Extraction with chloroform also generally resulted in moderate yields. Ethyl acetate and hexane extracted lower masses for most species. The dried extracts were resuspended in 10 ml of deionized water resulting in the extract concentrations [Table 1].

Qualitative phytochemical studies showed that methanol and water extracted the widest range of phytochemicals for both the fruits [Table 1]. All methanolic and aqueous extracts generally showed moderate to high levels of phenolics (both water soluble and insoluble phenolics), flavonoids, and saponins, as well as moderate levels of triterpenoids. Muntries and Illawarra plum also showed moderate levels of tannins. Low to moderate levels of alkaloids were also noted for the methanolic and aqueous extracts desert lime and bush tomato. The ethyl acetate extracts generally had similar phytochemical profiles as the methanolic and aqueous extracts, albeit at lower levels. Few phytochemical classes were noted in the chloroform or hexane extracts. As these tests generally screen for polar phenolic compounds, this is perhaps not surprising.

Table 1: The mass of dried extracted plant material, the concentration after resuspension, qualitative phytochemical screenings and antioxidant contents of fruit extracts

Plant species	Extract	Mass of dried extract (mg)	Resuspended extract concentration (mg/ml)	Total phenolics	Water soluble	Water insoluble	Cardiac glycosides	Saponins	Triterpenes	Polysteroids	Alkaloids (Meyer test)	Alkaloids (Wagner's test)	Flavonoids	Tannins	Free anthraquinones	Combined anthraquinones	Antioxidant capacity (mg AA equivalency)
Muntries	M	524	52.4	+++	+++	-	-	+++	++	-	-	-	+++	++	-	-	6.9
	W	350	35	+++	+++	+++	-	+++	++	-	-	-	+++	++	-	-	2.9
	E	19	1.9	+	-	+++	-	-	-	-	-	-	+	++	-	-	1.2
	C	120	12	+	-	-	-	-	-	-	-	-	+	-	-	-	0.4
	H	20	2	-	-	-	-	++	-	-	-	-	-	-	-	-	0.2
Illawarra plum	M	314	31.4	+++	+++	+++	-	+++	++	-	-	-	++	++	+	++	6.8
	W	195	19.5	+++	++	+++	-	+++	++	-	-	-	++	++	++	++	2.7
	E	3	0.3	+	-	+	-	-	-	-	-	-	++	+	-	-	1.2
	C	140	14	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3
	H	50	5	-	-	-	-	++	-	-	-	-	-	-	-	-	0.2
Native tamarind	M	107	10.7	+++	-	+++	-	++	-	-	-	-	+	-	-	-	9.2
	W	52	5.2	++	-	+++	-	-	-	-	-	-	+	-	++	-	8.4
	E	27	27	+	-	+	++	-	++	-	-	-	+	-	++	++	0.7
	C	120	12	-	-	-	+	++	-	-	-	-	-	-	-	-	0.2
	H	70	7	-	-	-	+	-	-	-	-	-	-	-	-	-	BDT
Lemon aspen	M	360	36	+++	-	-	+	-	++	-	-	-	+++	-	-	-	15.9
	W	162	16.2	+++	-	-	+	+++	++	-	-	-	+++	-	-	-	7.2
	E	66	6.6	+++	-	+	+++	-	++	-	-	-	-	-	-	-	6.4
	C	180	18	+++	-	+	+	-	-	-	-	-	-	-	-	-	6
	H	70	7	+	-	-	++	-	-	-	-	-	-	-	-	-	3.2
Desert lime	M	247	24.7	+++	++	++	+	-	++	-	-	++	+++	-	-	-	11.7
	W	182	18.2	+	-	-	+	++	++	-	-	-	+++	-	-	-	6.3
	E	4	0.4	+	-	-	-	-	++	-	-	+	++	-	-	-	1.1
	C	240	24	+	-	-	-	-	++	-	-	+	+	-	-	-	0.7
	H	140	14	-	-	-	-	++	-	-	-	+	-	-	-	-	0.3
Bush tomato	M	313	31.3	+++	-	+++	-	-	++	-	-	++	++	-	-	-	9.1
	W	79	7.9	+++	++	+++	-	+++	++	-	-	++	+++	++	-	-	5.6
	E	81	8.1	-	-	-	+	-	-	-	-	-	-	-	-	-	3.5
	C	280	28	+	-	-	+	-	++	-	-	+	+	-	-	-	3.7
	H	80	8	-	-	-	-	-	-	-	-	+	-	-	-	-	2.2

M: Methanolic extract; W: Aqueous extract; E: Ethyl acetate extract; C: Chloroform extract; H: Hexane extract; +++: A large response; ++: A moderate response; +: A minor response; -: No response in the assay, AA: Ascorbic acid, BDT: Below detection threshold

Antioxidant content

Antioxidant capacity (expressed as ascorbic acid equivalence) for the fruit [Table 1] ranged from below the level of detection to high of 15.9 mg ascorbic acid equivalence per gram of dried plant material extracted (lemon aspen fruit methanolic extract). The methanol extracts of all fruits and herbs had higher antioxidant capacities than the corresponding water, ethyl acetate, chloroform, or hexane extracts.

Antiproliferative activity

Aliquots of each extract were tested for the ability to block cell proliferation of HeLa and CaCo2 cell lines. Eighteen of the 30 fruit extracts tested displayed significant ($P < 0.01$) antiproliferative effects against HeLa cells [Figure 1]. A further seven extracts (lemon aspen, desert lime, and bush tomato aqueous extracts; Illawarra plum ethyl acetate extract; muntries, Illawarra plum, and desert lime hexane extracts) showed minor inhibition of HeLa cell proliferation although this inhibition was not significant ($P > 0.01$). All of the methanolic and chloroform extracts strongly inhibited HeLa cell growth. In contrast, only two of the aqueous extracts (muntries and Illawarra plum) significantly inhibited HeLa cell proliferation. Interestingly, three of the ethyl acetate extracts (muntries, native tamarind, and bush tomato) induced significant HeLa 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 was not evaluated in this study.

The extracts were similarly effective at inhibiting CaCo2 cancer cell proliferation [Figure 2]. Twenty-two of the 30 fruit extracts tested displayed significant ($P < 0.01$) antiproliferative effects against the CaCo2 cell line. As with HeLa cell proliferation, all methanolic extracts were effective inhibitors of CaCo2 cellular proliferation. Similarly, the aqueous extracts (with the exception of the native tamarind aqueous extract), ethyl acetate extracts (except the native tamarind and bush tomato extracts), and chloroform extracts were also effective inhibitors of CaCo2 proliferation. Of the hexane extracts, only the lemon aspen extract inhibited CaCo2 cell proliferation. All of the other hexane extracts (as well as the native tamarind water and bush tomato 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 some extracts was not evaluated in this study.

Table 2: The concentrations of extract required to achieve 50% reduction (IC50) or 25% reduction of HeLa and CaCo2 cell proliferation compared to the untreated control

Plant species	Extract	HeLa (μg/mL)		CaCo2 (μg/mL)	
		IC50	IC25	IC50	IC25
Muntries	M	-	6550	-	-
	W	-	4380	-	-
	E	-*	-*	-	-
	C	-	3144	-	-
	H	-	-	-*	-*
Illawarra plum	M	-	3925	-	4538
	W	-	2899	-	4135
	E	-	-	-	-
	C	-	-	-	-
	H	-	-	-*	-*
Native tamarind	M	1636	977	3309	2222
	W	-	-	-*	-*
	E	-*	-*	-*	-*
	C	-	-	-	-
	H	-	-	-*	-*
Lemon aspen	M	480	193	769	508
	W	-	-	885	623
	E	1560	1064	1301	732
	C	-	4218	-	3927
	H	-	-	-	6511
Desert lime	M	535	254	487	323
	W	-	-	-	3115
	E	-	-	487	343
	C	-	-	-	2989
	H	-	-	-*	-*
Bush tomato	M	1764	1309	5180	2169
	W	-	-	-	-
	E	-*	-*	-*	-*
	C	-	-	-	5548
	H	-	-	-*	-*

-*: No IC values were determined as proliferative activity was observed for the extract. Values indicate the mean IC50 or IC25 values for triplicate determinations. M: Methanolic extract; W: Aqueous extract; E: Ethyl acetate extract; C: Chloroform extract; H: Hexane extract. - (- indicates that): That IC50 or IC25 values were not achieved as inhibition did not exceed the relevant % at any concentration tested

The antiproliferative efficacy of the extracts against HeLa and CaCo2 cells was further quantified by determining the dose required to inhibit to 50% (IC50) or 25% of the control cell proliferation (IC25) [Table 2]. Lemon aspen and desert lime methanolic extracts displayed potent antiproliferative activity, with IC50 values against both cell lines <1000 μg/ml. The lemon aspen methanolic extract was particularly potent, with an IC50 value of 480 μg/mL against HeLa cells. Several other extracts (native tamarind methanolic extract, lemon aspen ethyl acetate extract, and bush tomato methanol) displayed moderate antiproliferative potency against both cell lines although their IC50 values was approximately an order of magnitude higher (IC50 <5000 μg/mL). Several extracts (lemon aspen aqueous extract, desert lime ethyl acetate extract) were potent inhibitors of only CaCo2 cell proliferation.

Quantification of toxicity

All extracts were initially screened undiluted in the assay [Figure 3]. For comparison, the reference toxin $K_2Cr_2O_7$ (1000 μg/ml) was also tested in the bioassay. The $K_2Cr_2O_7$ reference toxin was rapid in its onset of mortality, inducing nauplii death within the first 3 h of exposure and 100% mortality was evident following 4–5 h (results not shown). Similarly, most of the fruit extracts displayed >50% mortality rates at 24 h and 48 h.

To further quantify the effect of toxin concentration on the induction of mortality, the extracts were serially diluted in artificial seawater to test across a range of concentrations in the Artemia nauplii bioassay. Table 3 shows the LC50 values of the fruit extracts toward *A. franciscana*. No LC50 values are reported for the muntries aqueous and hexane extracts, the Illawarra plum and native tamarind hexane extracts or the desert lime methanolic and ethyl acetate extracts as <50% mortality was seen for all concentrations tested. All fruit extracts except native tamarind ethyl acetate extract were determined to be nontoxic with LC50 values much >1000 μg/ml following 24 h and exposure. Extracts with an LC50 of >1000 μg/ml toward Artemia nauplii have been defined as being nontoxic.[37]

High performance liquid chromatography-mass spectrometry quadrupole time-of-flight analysis

An aim of this study was to establish an HPLC-mass spectrometry (MS)/MS method for the metabolic profiling of the compounds in the bioactive extracts and to use this method to qualitatively differentiate and identify a significant number of these compounds. As the lemon aspen extracts displayed the greatest potency in the antiproliferative assay, the methanolic and aqueous methanol extracts were deemed the most promising extracts for further phytochemical analysis. Optimized HPLC-MS/MS parameters were developed and used to profile and compare the compound profiles from different extractions of lemon aspen fruit. The resultant total compound chromatograms for the positive-ion and negative-ion chromatograms of the methanolic extract are presented in Figure 4a and b, respectively. The positive-ion chromatogram had a significantly greater number of mass signal peaks detected. However, the negative-ion chromatogram had a higher base peak signal to noise ratio in the total ion chromatograms which may have hidden some peaks in the negative ionization mode.

Both the positive- and negative-ion lemon aspen fruit chromatograms of the methanolic extract revealed numerous peaks, particularly in the early and middle stages of the chromatogram corresponding to the elution of polar compounds. Nearly, all of the methanol extract compounds had eluted by 17 min (corresponding to approximately 50% acetonitrile). Indeed, multiple overlapping peaks eluted in the first 2 min with 5% acetonitrile. However, multiple peaks eluting later in the chromatogram, particularly evident in the negative ionization mode [Figure 4b] indicates the broad spread of polarities of the compounds in this extract.

The lemon aspen fruit aqueous extract in negative ionization mode [Figure 5a] also had large amounts of polar material eluting early in the chromatogram at similar elution volumes to many of the compounds in the methanol extract, although the aqueous extract in positive ion mode had a lesser amount and size of peaks corresponding to the mid polarity compounds in the middle of the chromatogram (10–20 min) at approximately 25–60% acetonitrile. Much fewer peaks were evident in the negative-ion chromatograms [Figure 5b]. The aqueous extract negative-ion chromatograms showed several major peaks, particularly at very early elution times. Many of these elution times correspond to peaks at similar elution times in the positive-ion chromatograms [Figure 5a], indicating the corresponding compounds eluting at these times may have functional groups that are capable of both gaining and losing electrons.

Qualitative mass spectral analysis of the lemon aspen fruit extracts

In total, 145 unique mass signals were noted for the lemon aspen fruit extracts [Table 4]. Putative empirical formulae were achieved for all of these compounds. Of the 145 unique molecular mass signals detected,

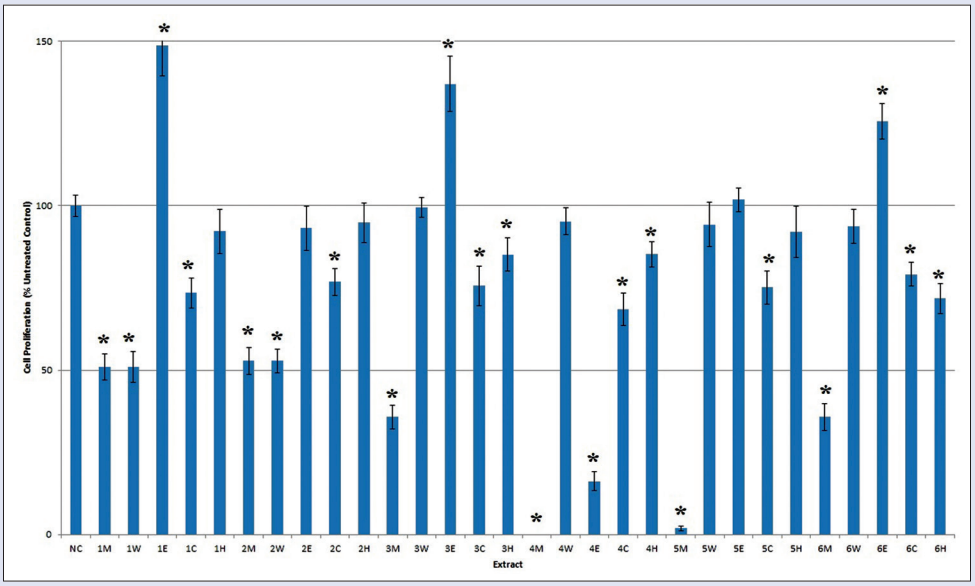

Figure 1: Antiproliferative activity of plant extracts and untreated controls against HeLa cancer cells measured as percentages of the untreated control cells. 1: Muntries fruit; 2: Illawarra plum fruit; 3: Native tamarind fruit; 4: Lemon aspen fruit; 5: Desert lime fruit; 6: Bush tomato fruit; M: Methanolic extract; W: Water extract; E: Ethyl acetate extract; C: Chloroform extract; H: Hexane extract; NC: Negative control. Results are expressed as mean percentages ± standard error mean of at least triplicate determinations. *Results that are significantly different to the untreated control ($P < 0.01$)

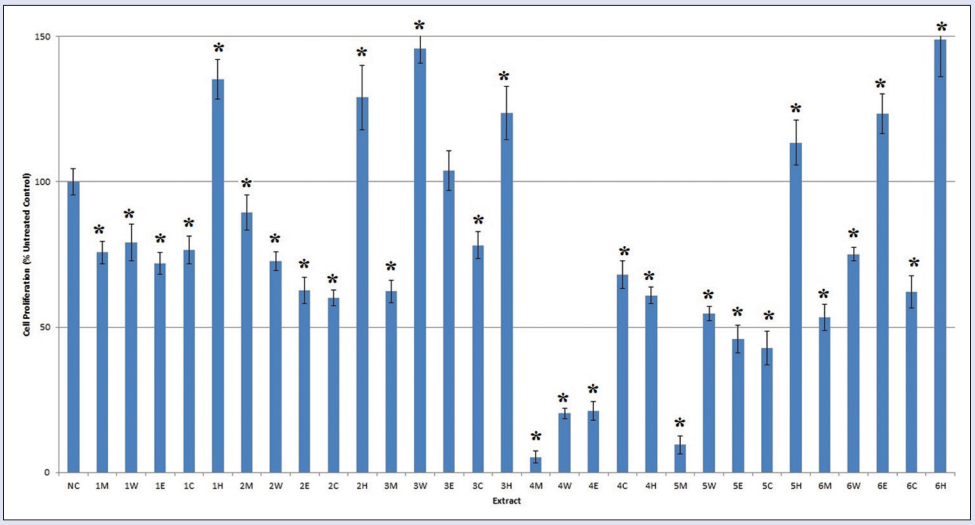

Figure 2: Antiproliferative activity of plant extracts and untreated controls against CaCo2 cancer cell lines measured as percentages of the untreated control cells. 1: Muntries fruit; 2: Illawarra plum fruit; 3: Native tamarind fruit; 4: Lemon aspen fruit; 5: Desert lime fruit; 6: Bush tomato fruit; M: Methanolic extract; W: Water extract; E: Ethyl acetate extract; C: Chloroform extract; H: Hexane extract; NC: Negative control. Results are expressed as mean percentages ± standard error mean of at least triplicate determinations. *Results that are significantly different to the untreated control ($P < 0.01$)

approximately 86 compounds were putatively identified by comparison against three accurate mass databases.

DISCUSSION

The antiproliferative efficacy of plant extracts of six Australian fruits was examined against two cancer cell lines *in vitro*; HeLa (cervical) and CaCo2 (colorectal). The observed antiproliferative activity correlated with the measured antioxidant capacity. The lemon aspen and desert lime methanolic extracts (15.9 and 11.9 mg ascorbic acid equivalents per original gram of fruit extracted, respectively) were determined to have the most potent antiproliferative activity, each with IC50 values <800 μg/ml against both cell lines. The extracts which displayed moderate antiproliferative potency against both cell lines (native tamarind methanolic extract, lemon aspen ethyl acetate extract, and bush tomato methanolic extract) also had high antioxidant capacities. Indeed, antioxidant capacities of 9.2, 6.4, and 9.1 mg ascorbic acid equivalents per original gram of fruit extracted were measured for each of these extracts, respectively. Conversely, many of the extracts which had low antioxidant activity (e.g. many of the ethyl acetate and hexane extractions) lacked antiproliferative activity. Indeed, an

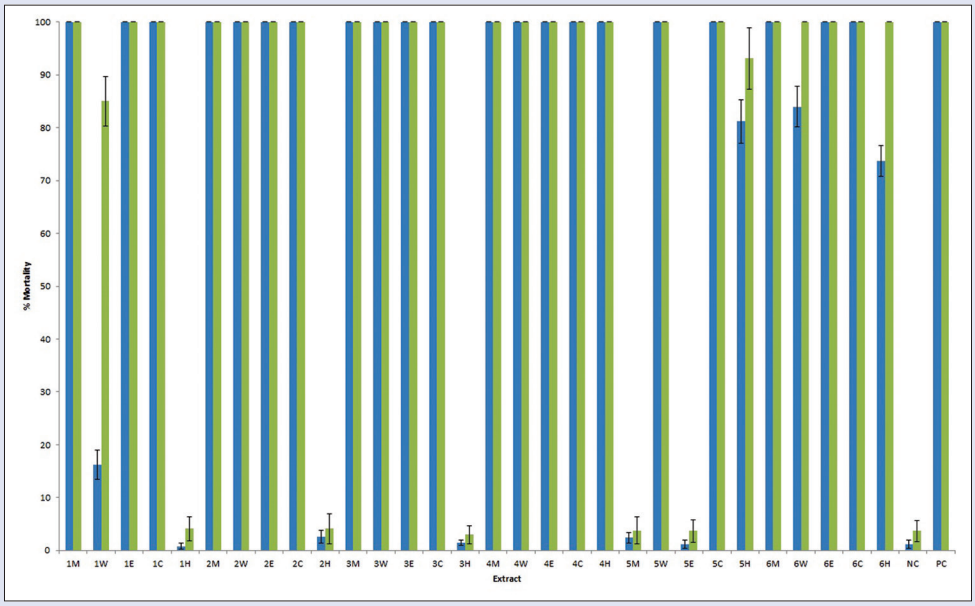

Figure 3: The lethality of the undiluted fruit extracts and control (1000 μg/mL) toward Artemia nauplii. Blue and green bars represent 24 h and 48 h mortality, respectively. 1: Muntries; 2: Illawarra plum; 3: Native tamarind; 4: Lemon aspen; 5: Desert lime; 6: Bush tomato; M: Methanolic extract; W: Water extract; E: Ethyl acetate extract; C: Chloroform extract; H: Hexane extract; NC: Negative (seawater) control; PC: Positive control (1000 μg/ml potassium dichromate). All tests were performed in at least triplicate, and the results are expressed as mean ± standard error mean

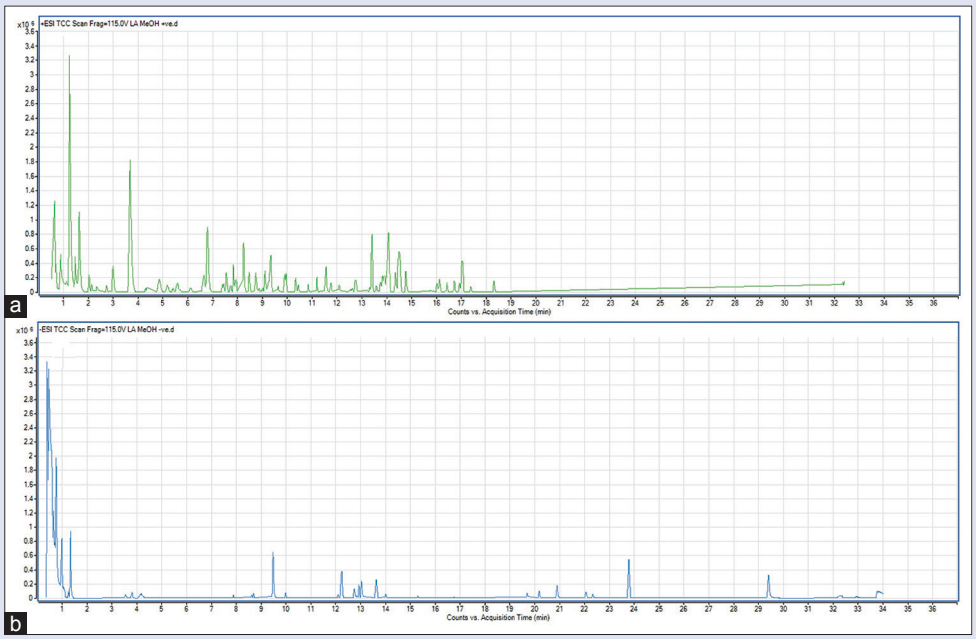

Figure 4: (a) Positive and (b) negative ion reverse phase-high performance liquid chromatography-mass spectrometry total compound chromatograms of 2 μl injections of lemon aspen methanolic extract

interesting trend was noted: The lower antioxidant ethyl acetate and hexane extracts not only failed to block cancer cell proliferation but also many were observed to stimulate proliferation in both HeLa cell samples and CaCo2 cell lines.

A previous study on the antioxidant activity of fresh apples reported a similar relationship between antioxidant activity and antiproliferative activity against CaCo2 cells.[38] It was suggested that the antiproliferative activity was due to the combination of phenolic acids and flavonoids. 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 strong antiproliferative activity, and at much lower levels, in the extracts that did not block cell proliferation. Flavonoids are believed to protect cells from disease by shielding

Figure 5: (a) Positive and (b) negative ion reverse phase-high performance liquid chromatography-mass spectrometry total compound chromatograms of 2 µl injections of lemon aspen aqueous extract

Table 3: The concentrations of the fruit extracts required to achieve 50% mortality (LC50) in the *Artemia franciscana* nauplii assay

Plant species	Extract	24 h LC50
Muntries	M	1965
	W	-
	E	1515
	C	7483
	H	-
Illawarra plum	M	1664
	W	1956
	E	1293
	C	5946
	H	-
Native tamarind	M	1595
	W	1862
	E	783
	C	2562
	H	-
Lemon aspen	M	1500
	W	1872
	E	1609
	C	1984
	H	5294
Desert lime	M	-
	W	3875
	E	-
	C	4495
	H	4883
Bush tomato	M	3467
	W	5372
	E	5704
	C	8510
	H	4062
NC		-
PC		186

Values indicate the mean LC50 for triplicate determinations. -: IC50 values were not achieved as inhibition did not exceed 50% at any concentration tested; M: Methanolic extract; W: Aqueous extract; E: Ethyl acetate extract; C: Chloroform extract; H: Hexane extract; NC: Negative control; PC: Positive control

lipids, proteins, and DNAs from oxidative damage.[39] Previous *in vitro* bioactivity studies of flavonoids have demonstrated anti-inflammatory, antioxidant, and anticancer activity.[39]

HPLC-MS-QTOF analysis putatively identified a number of compounds with antioxidant activity. Hydroxyethyl salicylate [Figure 6a],[40] ferulic acid [Figure 6b],[41] diftalone [Figure 6c],[42] gingerol [Figure 6d],[43] ketorolac glucuronide [Figure 6e],[44] rutin [Figure 6f],[45] theophylline [Figure 6g],[46] luteolin [Figure 6h],[47] diosmin [Figure 6i],[48] dihydrokaempferol [Figure 6j],[49] ellagic acid [Figure 6k],[50] trimethyl ellagic acid [Figure 6l],[50] and chlorogenic acid [Figure 6m][51] have all previously been identified as having strong antioxidant activity. The direct role of several of these compounds in anticancer mechanisms has also been demonstrated. Gingerol induces cell cycle arrest and cell death in the BxPC-3 pancreatic cancer cell line.[52] Ellagic acid induces apoptosis in HOS cells through the up-regulation of Bax and activation of caspase-3.[50] Chlorogenic acid induces cytotoxic activity against human oral squamous cell carcinoma-2 and human salivary gland tumor cell lines by activating caspase 3 and inducing nuclear condensation and DNA fragmentation.[53] Interestingly, this effect was found to be dose-dependent, with low concentrations of chlorogenic acid acting as an antioxidant and higher concentrations having prooxidant effects. Similarly, luteolin also has variable effects, functioning as either an antioxidant or a prooxidant at different concentrations.[54] That study also described several anticancer activities for luteolin including the induction of apoptosis and the inhibition of cell proliferation, metastasis, and angiogenesis.

A number of other compounds with known anticancer activity were also putatively identified in the lemon aspen extracts. Bicyclic acetals such as (1S,5R)-4-hydroxy-6,7-dioxabicyclo[3.2.1] oct-2-en-8-one [Figure 6n] have potent cytotoxic activity toward B-cell chronic lymphocytic leukemia.[55] Cantharidin [Figure 6o] has been shown to inhibit the proliferation of pancreatic cancer cells via oxidative stress-independent cell cycle arrest and the induction of apoptosis.[56] Naphthalenes structurally similar to

Table 4: Qualitative medium-pressure liquid chromatography-mass spectrometry quadrupole time-of-flight analysis of the lemon aspen fruit methanolic and aqueous extracts, elucidation of empirical formulae, and putative identification (where possible) of the compounds

Putative Identification	Empirical formula	Molecular mass	Retention time	Methanol extract	Aqueous extract
Acetic anhydride	C4 H6 O3	102.0328	0.478	–	
Quinone	C6 H4 O2	108.0217	1.603	+	+
3-furoic acid	C5 H4 O3	112.0161	0.387		–
Purine	C5 H4 N4	120.0436	32.377	+	
phloroglucinol	C6 H6 O3	126.0322	1.603	+	+
2-deoxy-D-ribose	C5 H10 O4	134.0584	0.45		–
m-nitrotoluene	C7 H7 N O2	137.047	0.521	+	
(1S,5R)-4-hydroxy-6,7-dioxabicyclo[3.2.1] oct-2-en-8-one (isomer 1)	C6 H6 O4	142.0269	3.647	+	
(1S,5R)-4-hydroxy-6,7-dioxabicyclo[3.2.1] oct-2-en-8-one (isomer 2)	C6 H6 O4	142.027	1.231	+	
(E)-2-methylglutaconic acid	C6 H8 O4	144.0418	0.714	–	
Xylitol	C5 H12 O5	152.0699	0.44		–
	C4 H4 N4 O3	156.0286	0.617	+	
Oxanamide	C8 H15 NO2	157.1109	0.614	+	+
2-oxoadipic acid	C6 H8 O5	160.0376	1.229	+	
Ethyl (ethylperoxy) (oxo) acetate	C6 H10 O5	162.0528	2.116	+/–	–
Phthalate	C8 H4 O4	164.0111	3.106		+
	C9 H11 N O2	165.0768	1.451	+	
Ethyl 4-hydroxybenzoate	C9 H10 O3	166.0635	0.956	–	
(1S,5R)-4-Oxo-6,8-dioxabicyclo[3.2.1] oct-2-ene-2-carboxylic acid	C7 H6 O5	170.0219	1.216	+	
Dehydroascorbic acid (oxidized Vitamin C)	C6 H6 O6	174.0164	0.384		–
Shikimic acid	C7 H10 O5	174.0532	3.646	+	
2-hydroxyethyl salicylate	C9 H10 O4	182.0578	0.742	–	
3,4,5-trihydroxybenzoic acid monohydrate	C7 H8 O6	188.0326	1.219	+	
Citric acid	C6 H8 O7	192.0268	0.749	–	+/–
Hydroxy-7-methyl-4H,5H-pyrano[4,3-b] pyran-dione	C9 H6 O5	194.0221	2.001	+	+
Ferulic acid	C10 H10 O4	194.0582	1.222	–	
Feroxidin	C11 H14 O3	194.0948	11.548	+	
Cantharidin	C10 H12 O4	196.074	1.309	–	
	C8 H10 O6	202.0478	3.645	+	
Calamenene (isomer 1)	C15 H22	202.1722	13.379	+	
Calamenene (isomer 2)	C15 H22	202.1724	16.403	+	
2,3-O-(oxymethylene) hexopyranose	C7 H10 O7	206.0432	1.22	+/–	+
Cyclazodone	C12 H12 N2 O2	216.0901	5.567	+	+
5-hydroxycalamenene	C15 H22 O	218.1669	12.582	+	
	C8 H12 O7	220.0589	3.645	+/–	
	C9 H8 N4 O3	220.059	2.971	+	
Spathulenol (isomer 1)	C15 H24 O	220.1828	13.575	+	
Spathulenol (isomer 2)	C15 H24 O	220.1831	15.998	+	
Spathulenol (isomer 3)	C15 H24 O	220.1832	10.321	+	
Gladiolic acid	C11 H10 O5	222.0532	9.873	+	+
	C7 H13 N O7	223.0696	1.228	+	
	C13 H10 N2 O2	226.0746	8.511		+
Heptyl heptanoate	C14 H28 O2	228.2098	20.927		–
(2R,3S)-3-(3-carboxylatepropanoyl)-5-oxotetrahydro-2-furancarboxylic	C9 H8 O7	228.0251	1.23	+	
Ozagrel	C13 H12 N2 O2	228.09	7.84	+	+
Heptyl heptanoate	C14 H28 O2	228.2093	20.899	–	
Metomidate	C13 H14 N2 O2	230.1059	7.704	+	+
	C9 H14 O7	234.0746	7.91	+	
Drimenin	C15 H22 O2	234.1624	16.105	+	
Capsidiol (isomer 1)	C15 H24 O2	236.1779	11.496	+	
Capsidiol (isomer 2)	C15 H24 O2	236.178	17.371	+	
Capsidiol (isomer 3)	C15 H24 O2	236.1781	14.516	+	
	C12 H19 N3 O2	237.1479	7.076		+
Kessil alcohol	C15 H26 O2	238.1936	14.479	+	
	C12 H5 N3 O3	239.0323	2.97	+	
Isopentanoic acid	C15 H30 O2	242.2247	22.342	–	–
	C7 H4 N2 O8	243.999	1.227	+	
Leu Leu	C12 H24 N2 O3	244.1794	7.424	+	+

Contd...

Table 4: Contd...

Putative Identification	Empirical formula	Molecular mass	Retention time	Methanol extract	Aqueous extract
Nodakenetin	C14 H14 O4	246.0897	12.068	+	
Acetyltryptophan	C13 H14 N2 O3	246.1007	5.157	+	
Nitrefazole	C10 H8 N4 O4	248.0538	0.737		+
	C15 H26 O3	254.1881	16.722	+	
7-palmitoleic acid	C16 H30 O2	254.225	22.059	–	–
11-keto pentadecanoic acid	C15 H28 O3	256.2042	13.987	–	
Palmitic acid	C16 H32 O2	256.241	23.786	–	–
	C10 H16 O8	264.085	0.64		–
Diftalone	C16 H12 N2 O2	264.0913	10.429	+	
Propacetamol	C14 H20 N2 O3	264.1482	3.692	+	+
	C9 H14 O9	266.0642	0.737		+/–
Lauroylsarcosine	C15 H29 N O3	271.2155	13.978	+	
Lauroylsarcosine	C15 H29 N O3	271.2155	17.008	+	
	C12 H11 N5 O3	273.0857	0.686	+	
Macowine	C16 H19 N O3	273.1372	8.452	+	+
2,15-dihydroxy-pentadecylic acid	C15 H30 O4	274.2148	9.96	–	
	C9 H13 N11	275.1351	0.864	+	
Methyl salicylate	C17 H24 O3	276.1709	17.046	+	
Queuine	C12 H15 N5 O3	277.1171	0.556		+
7-hydroxy-10E,16-heptadecadien-8-ynoic acid	C17 H26 O3	278.1861	14.479	+	
Dihydroartemisinin	C15 H24 O5	284.1631	12.734	+/–	
Hexyl dodecanoate	C18 H36 O2	284.2723	26.727		–
N, N-dimethylchlorpromazine	C15 H15 Cl N2 S	290.0648	0.655		+
	C9 H15 N11 O	293.146	0.863	+	
Gingerol	C17 H26 O4	294.184	13.005	–	–
Sulazepam	C16 H13 Cl N2 S	300.0483	6.089	+	+/–
Tegaserod (isomer 1)	C16 H23 N5 O	301.1893	2.708	+	
Tegaserod (isomer 2)	C16 H23 N5 O	301.1895	12.731	+	
	C14 H8 O8	304.0206	0.735		+
	C12 H18 O9	306.0954	0.636		–
Pyrethrosin	C17 H22 O5	306.1469	15.328		+
Propentofylline	C15 H22 N4 O3	306.1705	8.22	+	+
2,2'-(Methylenebis[oxy]) bis (ethylmalonic acid)	C11 H16 O10	308.0752	0.647		+
Dantrolene	C14 H10 N4 O5	314.0643	8.988	+	
	C16 H29 N O5	315.2053	7.365	+	
9,13-dihydroxy-11-octadecenoic acid	C18 H34 O4	314.2464	20.177	–	–
Butalamine	C18 H28 N4 O	316.2259	12.903	–	
Denbufylline	C16 H24 N4 O3	320.1855	7.567	+	
	C15 H24 N4 O4	324.1801	1.669	+	
	C13 H10 N8 O3	326.0883	0.408		–
	C11 H22 N10 O2	326.1922	19.696	–	–
	C15 H21 N O7	327.1323	1.521	+	
	C13 H12 N4 O7	336.0704	0.614		+
	C16 H18 O8	338.1006	3.52	–	
Clorotepine	C19 H21 Cl N2 S	344.1114	4.138	–	
Granisetron metabolite 1	C18 H24 N4 O3	344.1846	12.218	–	
	C13 H14 N4 O8	354.0813	0.401	–	–
Oxypurinol-7-ribonucleotide	C10 H13 N4 O9 P	364.0418	0.623		+
	C14 H16 N4 O8	368.0972	0.529	–	
9-(5-O-benzoylribofuranosyl)-3,9-dihydro-6H-purin-6-one	C17 H16 N4 O6	372.1058	3.787	–	
Sarafloxacin	C20 H17 F2 N3 O3	385.1218	0.996	+	
	C15 H38 N10 O2	390.3181	29.397	–	–
	C13 H8 N6 O9	392.0366	0.57	+/–	+
	C24 H10 N2 O5	406.0575	0.538	–	
	C12 H20 O16	420.0728	0.637	–	
	C16 H14 N4 O10	422.0703	1.499		+
Ketorolac glucuronide	C21 H21 N O9	431.1205	0.601		+
Propranolol glucuronide	C22 H29 N O8	435.1906	7.811	+	
	C17 H21 N5 O9	439.1341	0.685		+
	C13 H20 O17	448.0678	0.601		–
	C15 H23 N O15	457.1074	1.766		+

Contd...

Table 4: Contd...

Putative Identification	Empirical formula	Molecular mass	Retention time	Methanol extract	Aqueous extract
Trans-1,4-bis (2-chlorobenzaminomethyl) cyclohexane dihydrochloride	C22 H30 Cl4 N2	462.117	12.726	–	
	C17 H20 O15	464.0807	0.926		+
	C19 H6 N4 O11	466.005	1.241	+	
	C22 H28 N8 O4	468.223	4.834	+	
	C22 H28 N8 O4	468.2235	7.519	+	+
Chlortetracycline	C22 H23 Cl N2 O8	478.1121	12.068	–	
	C17 H22 O16	482.091	0.914		+
	C18 H20 N4 O13	500.1031	0.341		–
	C17 H27 N O17	517.1281	0.931		+
	C19 H14 N10 O9	526.0945	0.557		+
	C23 H50 N10 O4	530.4014	28.412		+
	C23 H22 O16	554.0902	0.544	+	
	C29 H39 N9 O3	561.3177	11.174	+	
Apiin	C26 H28 O14	564.1485	9.609	+/–	+/–
	C26 H16 N8 O8	568.1099	0.61	–	
	C32 H35 N9 O2	577.2921	14.062	+/–	+/–
Glucofrangulin	C27 H30 O14	578.1651	9.915	+	+
	C25 H18 N14 O5	594.1582	8.718	+	+
	C28 H26 N4 O11	594.1603	9.097	+/–	
8'-hydroxydihydroergotamine	C33 H37 N5 O6	599.2729	14.067	+	+
Rutin	C27 H30 O16	610.154	8.777	+	+
	C29 H28 N18 O	644.27	10.823	+	
	C28 H20 N2 O17	656.0766	1.225	+	
	C34 H44 N6 O8	664.322	13.165	+/–	
	C53 H54 O2	722.4123	15.728	+/–	
	C31 H26 N24	734.2773	11.548	+	

+ and –: The mass spectral mode in which that the molecule was detected

calamanene [Figure 6p] and hydroxycalamanene [Figure 6q] have been associated with the induction of apoptosis in human lung cancer cell lines by upregulating DR4 and DR5 cell death receptors and enhancing the activation of caspases 3, 7, 8, and 9.[57] However, other reports also indicate that naphthalenes may be mildly carcinogenic themselves.[58] Lauroyl sarcosinate [Figure 6r] may function as an antagonist of sarcosine, which is produced during prostate cancer progression, and induces the prostate cells into an invasive phenotype. Previous studies have shown that sarcosine (but not its structural isomers) induces an increase in human epithelial growth factor receptor 2 mRNA levels.[59] If lauroyl sarcosinate antagonizes the effects of sarcosine, it may have anticancer effects. Queuine [Figure 6s] administration to DLAT cancerous mice activates cellular enzymatic antioxidant defenses, blocking oxidative stress and tumorigenesis.[60] Dihydroartemisinin [Figure 6t] has recently been shown to have anticancer activity in several cell lines, including BxPC-3 and AsPC-1 pancreatic cancer cell lines[61] and ovarian cancer cells.[62] In the pancreatic carcinoma lines, dihydroartemisinin inhibited cell proliferation by down-regulating the expression of proliferating cell nuclear antigen and cyclin D1 and up-regulating p21.[61] The same study also identified several apoptotic mechanisms for dihydroartemisinin including the induction of a reduction in the Bcl-2/Bax ratio and induction of caspase-9 activity.

The findings reported here also demonstrate that the majority of the fruit extracts were nontoxic toward *A. franciscana* nauplii. Extracts with LC50 values >1000 μg/ml toward Artemia nauplii have been defined as being nontoxic.[37] Only the native tamarind fruit ethyl acetate extract displayed an LC50 values below 1000 μg/mL. Therefore, all other extracts were determined to be nontoxic. Despite being considered toxic, the LC50 of the native fruit ethyl acetate extract (783 μg/ml) would classify this extract as low to moderate toxicity. Furthermore, native tamarind fruits have previously been reported to have high vitamin C contents. As Artemia nauplii are susceptible to pH changes,[37] 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.

CONCLUSIONS

The results of this study demonstrate the potential of several extracts prepared from high antioxidant Australian fruits to block the growth of HeLa and CaCo2 cell lines. In particular, lemon aspen methanolic and aqueous extracts were identified as particularly potent inhibitors of cell proliferation. Furthermore, these extracts displayed low toxicity in the Artemia nauplii bioassay, indicating their therapeutic potential. A number of interesting compounds with activities associated with anticancer activities were identified by LC-MS. However, the antiproliferative mechanisms of the extracts were not examined in this study. Further studies aimed at identifying the antiproliferative mechanisms of these extracts are needed.

Acknowledgments

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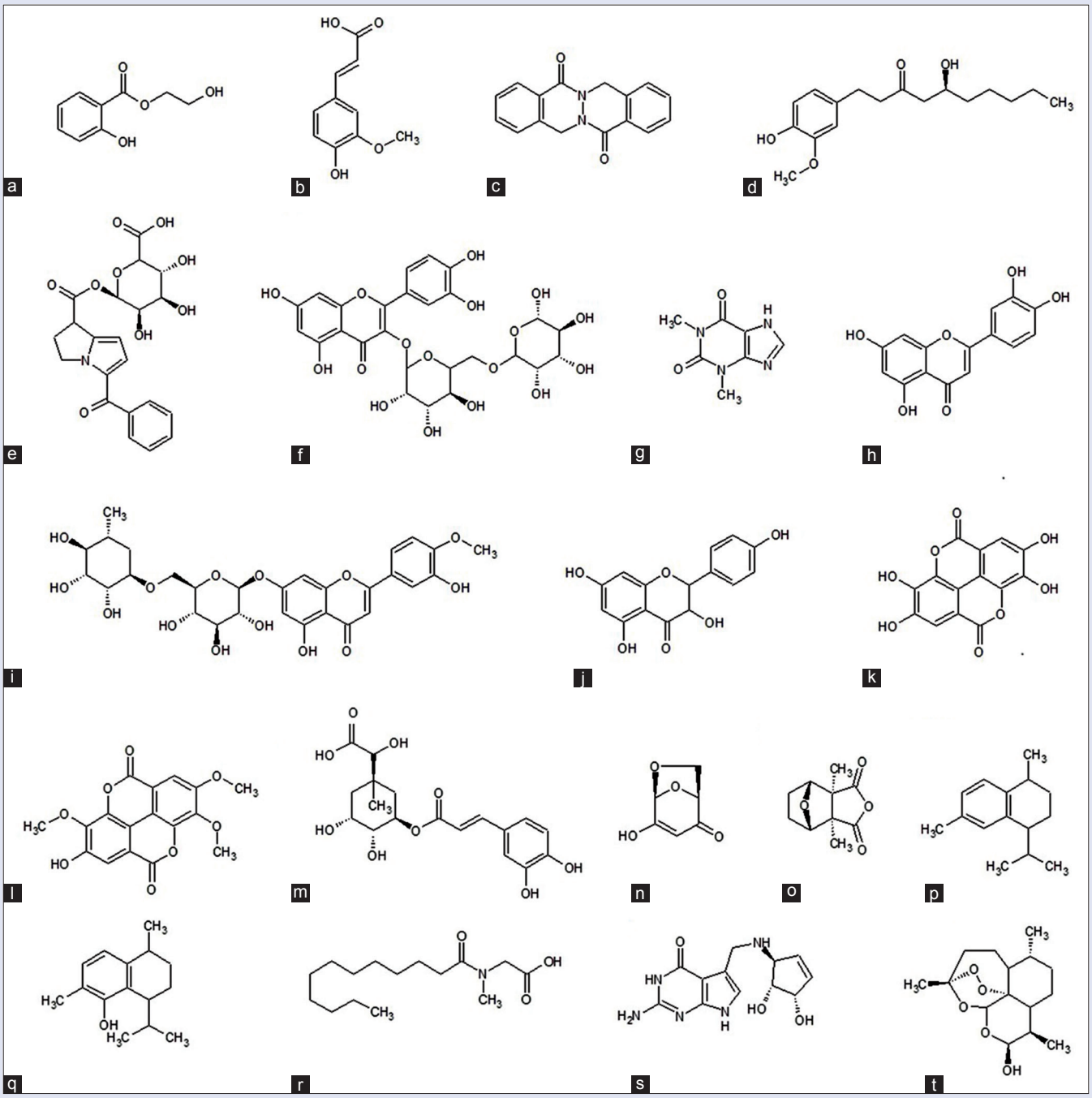

Figure 6: Chemical structures of lemon aspen fruit compounds detected in the methanolic and aqueous extracts (a) hydroxyethyl salicylate; (b) ferulic acid; (c) diftalone; (d) gingerol; (e) ketorolac glucuronide; (f) rutin; (g) theophylline; (h) luteolin; (i) diosmin; (j) dihydrokaempferol; (k) ellagic acid; (l) trimethyl ellagic acid; (m) chlorogenic acid; (n) (1S,5R)-4-hydroxy-6,7-dioxabicyclo[3.2.1]oct-2-en-8-one; (o) cantharidin; (p) calamanene; (q) hydroxycalamanene (r) lauroyl sarcosinate; (s) queuine; (t) dihydroartemisinin

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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