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Original Article

Cakile maritima Scop. Extracts Inhibit CaCo2 and HeLa Human Carcinoma Cell Growth: GC-MS Analysis of an Anti-Proliferative Extract

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ABSTRACT

Introduction: Exposure to high levels of antioxidants has been linked to the treatment and prevention of some cancers. Although *Cakile maritima* has a high antioxidant capacity, it is yet to be tested for the ability to inhibit the proliferation of cancer cells. **Methods:** Solvent extracts prepared from *C. maritima* plant material were analysed for antioxidant capacity by the DPPH free radical scavenging assay. Anti-proliferative activities against CaCo2 and HeLa cancer cells were determined by an MTS based cell proliferation assay. Toxicity was determined by the *Artemia franciscana* bioassay. The most potent anti-proliferative extract (hexane) was further investigated using non-targeted GC-MS headspace analysis. **Results:** Good DPPH radical scavenging activity was calculated for all *C. maritima* extracts. The methanolic and ethyl acetate extracts had particularly strong antioxidant activity (IC_{50} of 4.7 and 3.4 µg/mL respectively). Interestingly, the hexane extract which had the lowest DPPH radical scavenging activity (IC_{50} 13.6 µg/mL), was the most potent inhibitor or CaCo2 and HeLa carcinoma cell growth, with IC_{50}'s of 12 and 126 µg/mL respectively. The ethyl acetate extract was also a potent inhibitor of proliferation (IC_{50} values of 185 and 468 µg/mL against CaCo2 and HeLa, respectively). The methanolic extract (IC_{50} values of 2261 and 2046 µg/mL against CaCo2 and HeLa respectively) displayed only moderate anti-proliferative activity, demonstrating that antioxidant activity did not correspond with anti-proliferative activity. All of the extracts were determined to be nontoxic in the *Artemia franciscana* bioassay, with LC_{50} values substantially >1000 µg/mL. Non-biased GC-MS headspace analysis of the *C. maritima* hexane extract highlighted several interesting compounds that may contribute to the therapeutic bioactivities of the extract. **Conclusion:** The lack of toxicity and the anti-proliferative activity of the hexane and ethyl acetate *C. maritima* extracts against HeLa and CaCo2 cancer cell lines indicates their potential in the treatment and prevention of some cancers.

Key words: Brassicaceae, European searocket, Antioxidant, Oxidative stress, Caco2, HeLa, Anticancer activity.

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INTRODUCTION

Regulation of cellular redox state is important to maintain the function and well-being of individual cells and of the whole organism. When cellular redox regulatory mechanisms are unable to cope effectively with the production of Reactive oxygen species (ROS), the cell is placed under oxidative stress and the ROS may induce significant damage to cellular proteins and lipids, thereby negatively influencing the cells survival. Excessive oxidative stress and the inability to overcome it has been implicated in numerous diseases including atherosclerosis diabetes, cirrhosis,[1] autoimmune disease and chronic inflammation,[2] neurodegeneration[1] and cancer.[3] The relationship between cellular redox state and cancer progression has been particularly well studied and the induction of cellular oxidative stress has been linked with several types of cancer.[4,5] As well as damaging cellular proteins and lipids, ROS may also induce the formation of DNA adducts which may promote carcinogenic activity. An understanding of cellular redox regulation is important for the identification of new targets for the development of drugs to prevent and treat cancers.

Cells regulate their redox state by balancing the levels of oxidants and antioxidants by both enzymatic and non-enzymatic mechanisms. The antioxidant defensive enzymes include catalase, thioredoxin, thioredoxin reductase, superoxide dismutase, glutathione reductase and glutathione peroxidase.[6] The non-enzymatic antioxidant defences include glutathione, as well as vitamins A, C and E. Interestingly, high dietary intakes of non-enzymatic antioxidants decrease the incidence of chronic diseases including some cancers.[7] High antioxidant herbal medicines

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and culinary plants contain multiple phenolic compounds that are strong antioxidants.[8] Many of these compounds can scavenge ROS, protect cell constituents against oxidative damage and thereby prevent carcinogenesis and retard the disease's progression. Thus, high antioxidant plant extracts may be useful for both preventing and treating some cancers.

Cakile maritima Scop. (Family Brassicaceae; commonly known as European searocket) is a herb which is native to Europe and the northern parts of Africa. It can survive in harsh conditions, thrives in sandy, coastal regions, often displaces native local taxa and has become widely naturalised internationally. *C. maritima* grows to 40 cm in height and has a multi-branched stem. All parts of the plant have a high antioxidant capacity and are particularly rich in ascorbic acid.[9] The entire plant is consumed, particularly when other food sources are scarce.[10] It is most frequently used as a flavouring agent, although young raw leaves may also be eaten fresh in salads. The powdered *C. maritima* roots can also be used to make bread. Furthermore, its high antioxidant capacity provides *C. maritima* with medicinal potential as high dietary intake of non-enzymatic antioxidants has been linked with decreased incidence of some chronic diseases, including some cancers, chronic inflammation, atherosclerosis and Alzheimer's disease.[1,11] Indeed, individuals with elevated dietary intakes of non-enzymatic antioxidants such as vitamins A, C and E are less likely to suffer from some diseases including cancer and chronic inflammation.[1] Furthermore, several studies have demonstrated bacterial growth inhibitory and anti-inflammatory activities for several culinary plants with high antioxidant capacities and have linked the bioactivities to their free radical scavenging activities.[12-16] A recent study from our group also reported good antibacterial activity for *C. maritima* whole plant extracts.[17] Despite this, we were unable to find reports examining the anticancer activity of *C. maritima.*

MATERIALS AND METHODS

Plant source and extraction

The aerial parts of *Cakile maritima* Scop. were collected and identified by Dr. Ahmed M. Abd El-Gawad, from Kafr-Saad, Damietta Governorate, Egypt in April 2015. A voucher specimen (Code No: CAM-311-015) was deposited at the herbarium of the Botany Department, Faculty of Science, Mansoura University, Egypt. The *C. maritima* aerial parts were air-dried in the shade and 1 kg of dried plant material were subsequently pulverized to a coarse powder. The powder was macerated in 70% methanol (5 L) at room temperature for five days, filtered and evaporated under vacuum, yielding a dark resinous extract (58 g). The dried extract was dissolved in 1L of distilled water and successively fractionated using *n*-hexane, ethyl acetate and methanol respectively (each in 0.5L of solvent, three times). Each fraction was separately dried under vacuum to yield 10.5, 15.0 and 32.5 gm of dried extracted material respectively

Qualitative phytochemical studies

The extracts were analysed for the presence of saponins, phenolic compounds, flavonoids, phytosterols, triterpenoids, cardiac glycosides, anthraquinones, tannins and alkaloids by previously described assays.[18-20]

Antioxidant capacity

The antioxidant capacity of each sample was assessed using a modified DPPH free radical scavenging method.[17] Briefly, DPPH solution was prepared as a 400 µM solution by dissolving DPPH (Sigma, Australia) in AR grade methanol (Ajax, Australia). The *C. maritima* extracts were diluted in methanol across the concentration range 10-80 µg/mL. Aliquots (20 µL) of each extract were mixed with 80 µL of 100 mM Tris-HCl buffer (pH 7.4). Ascorbic acid was prepared fresh and examined across the range 0-25 µg per well as a reference. A volume of 100 µL of DPPH solution was added to each well to give a volume of 250 µL. Blanks for each extract or ascorbic acid concentration, were also included on each plate. The assay mixtures were incubated in the dark for 20 min at 23°C and the absorbance were recorded at 515 nm using a Molecular Devices, Spectra Max M3 plate reader. All tests were performed in triplicate and triplicate controls were included on each plate. The % DPPH decolourisation was calculated using the following formula:

$$\% \text{ decolorisation} = [1-(A_{test}/ A_{control})] \times 100$$

IC_{50} values were determined as the test concentration capable of scavenging 50 % of the DPPH radicals via linear regression.

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 µg/mL streptomycin, 50 IU/mL penicillin, 2 mM glutamine and 10 % foetal 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 anti-proliferative activity

Anti-proliferation of the extracts was assessed as previously described.[21] Briefly, 70 µL of cell culture containing approximately 5000 cells was added to the wells of a 96 well plate and 30 µL of the test extracts or cell media (for the negative control) was added to individual wells. The plates were then incubated at 37°C, 5% CO_2 for 12 h in a humidified atmosphere. A volume of 20 µL of Cell Titre 96 Aqueous One solution (Promega) was added to each well and the plates were incubated for a further 3 h. The absorbance was measured at 490nm using a Molecular Devices, Spectra Max M3 plate reader. All tests were performed in at least triplicate, with 3 internal repeats (n=9) and triplicate controls were included on each plate. The anti-proliferative 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

Potassium dichromate ($K_2Cr_2O_7$) (AR grade, Chem-Supply, Australia) was prepared in deionised water (4 mg/mL) and serially diluted in artificial seawater for use as a reference toxin. Toxicity of the *C. maritima* extracts, the reference toxin and the conventional antibiotics was assessed using a modified *Artemia franciscana* nauplii lethality assay.[22,23] The LC_{50} with 95% confidence limits for each treatment was calculated using probit analysis.

Non-targeted GC-MS head space analysis

Chromatographic separations were performed using a Shimadzu GC-2010 plus (USA) linked to a Shimadzu MS TQ8040 (USA) mass selective detector system as previously described.[24] The system was equipped with a Shimadzu auto-sampler AOC-5000 (USA) fitted with a Supelco (USA) divinyl benzene/carbowax/polydimethylsiloxane (DVB/CAR/PDMS) solid phase micro-extraction fibre (SPME) handling system. Chromatographic separation was accomplished using a 5% phenyl, 95% dimethylpolysiloxane (30m x 0.25mm id x 0.25µm) capillary column

(Restek USA). Helium was employed as a carrier gas at a flow rate of 0.79 mL/min and an injector temperature of 230°C. Sampling utilised a SPME cycle for a period of 10 min exposure to allow for absorption. The SPME was then desorbed in the injection port for 1 min at 250°C. The initial column temperature was held at 30°C for 2 min, increased to 140°C for 5 min, then increased to 270°C over a period of 3 mins and held at that temperature for the duration of the analysis. The GC-MS interface was maintained at 200°C. No signal was acquired for the first minute following sample injection and the system was run in split-less mode. The mass spectrometer was operated in the electron ionisation mode at 70eV and the mass signals were recorded in total ion count (TIC) mode. Data acquisition began 1 min after injection and continued for 45 mins utilising a mass range of 45-450 m/z.

Statistical analysis

Data are expressed as the mean ± SEM of at least three independent experiments, each with internal triplicates (n=9). 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, qualitative phytochemical screening and antioxidant capacity

Extraction of 1 kg of *C. maritima* whole plant with 5L of 70% methanol yielded 58 g (i.e. 5.8 % yield) of dried total extract (TE) (Table 1). Sequential extraction with hexane, ethyl acetate and methanol yielded 10.5 g (approximately 1.1% yield), 15.0 g (approximately 1.5 % yield) and 32.5 g (approximately 3.3 % yield) of total extracted material respectively. The dried extracts were resuspended in deionised water (containing 1% DMSO) to give the extract concentrations shown in Table 1.

All extracts contained a wide range of phytochemical classes (Table 1). Phenolics and flavonoids were present in moderate-high levels in the ethyl acetate and methanolic extracts. Triterpenoids were present in all extracts, albeit in low relative abundance. Saponins were present in methanolic and ethyl acetate extracts in low relative abundance (but not in the hexane extract), whilst phytosterols were present in the methanolic and hexane extracts (but not in the ethyl acetate extract). Alkaloids were only present in the methanolic extract, albeit in low abundance. All extracts were completely devoid of cardiac glycosides, tannins and anthraquinones. Antioxidant capacity (expressed as the concentration capable of scavenging 50% of the DPPH radical) for the *C. maritima* extracts (Table 2) ranged from a DPPH radical scavenging IC_{50} of 3.4 µg/mL (ethyl acetate *C. maritime* extract) to a high of 13.6 µg/mL (*C. maritima* hexane extract).

Anti-proliferative activity

Aliquots of all *C. maritima* extracts were tested for the ability to block cell proliferation of $CaCo_2$ and HeLa cell lines. All *C. maritima* extracts tested displayed significant (p<0.01) anti-proliferative effects against CaCo2 cells (Figure 1). The hexane extract was particularly potent and completely inhibited CaCo2 proliferation. The methanolic and ethyl acetate extracts were also potent inhibitors of CaCo2 proliferation, inhibiting cellular proliferation by approximately 95 % compared to the untreated control CaCo2 cell proliferation. The *C. maritima* extracts were similarly effective at inhibiting HeLa cancer cell proliferation, with all *C. maritima* extracts displaying potent inhibitory activity (Figure 2). In contrast to CaCo2 proliferation, the mid polarity ethyl acetate extracts was the most effective inhibitor of HeLa proliferation (99 % inhibition of proliferation). The methanolic and hexane extracts were also effective inhibitors of HeLa proliferation, decreasing growth by approximately 85 % and 95 % of the untreated control HeLa cell proliferation respectively.

The anti-proliferative efficacy of the extracts against CaCo2 and HeLa cells was further quantified by determining the dose required to inhibit to 50 % (IC_{50}) of the control cell proliferation (Table 2). The calculated IC_{50} values indicate that the order of efficacy for the extracts was hexane extract > ethyl acetate extract > methanol extract against both cell lines. The hexane (IC_{50} 12 and 126 µg/mL against CaCo2 and HeLa cells

Table 1: The mass of dried extracted material, the concentration after resuspension in deionised water, qualitative phytochemical screenings and antioxidant capacities of the *C. maritima* extracts.

Extract	Mass of Dried Extract (g)	Concentration of Resuspended Extract (mg/mL)	Antioxidant Capacity measured as IC_{50} (µg/mL)	Total Phenolics	Water Soluble Phenolics	Water Insoluble Phenolics	Cardiac Glycosides	Saponins	Triterpenes	Phytosteroids	Alkaloids (Mayer Test)	Alkaloids (Wagner Test)	Flavonoids	Tannins	Free Anthraquinones	Combined Anthraquinones
TE	58	58	6.8	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CMM	32.5	40	4.7	+++	+++	++	-	+	+	+	+	+	+++	-	-	-
CME	15.0	10	3.4	+++	++	++	-	+	+	-	-	-	++	-	-	-
CMH	10.5	6	13.6	++	+	+	-	-	+	+	-	-	-	-	-	-

+++ indicates a large response; ++ indicates a moderate response; + indicates a minor response; - indicates no response in the assay; NT = not tested. CMM = methanolic *C. maritima* extract; CME = ethyl acetate *C. maritima* extract; CMH = hexane *C. maritima* extract. Antioxidant capacity was determined by DPPH reduction and is expressed as the test concentration capable of scavenging 50 % of the DPPH radical.

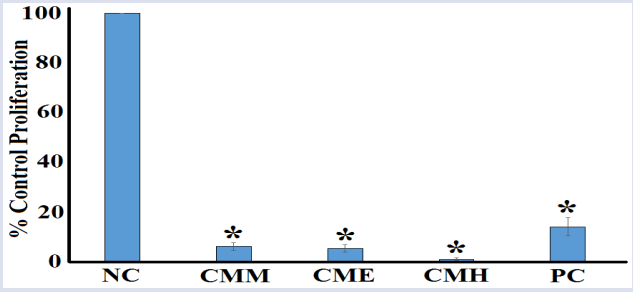

Figure 1: Anti-proliferative activity of the *C. maritima* extracts and untreated controls against Caco2 cancer cells measured as percentages of the untreated control cells. NC = untreated cell growth (negative control); CMM = *C. maritima* methanolic extract; CME = *C. maritima* ethyl acetate extract; CMH = *C. maritima* hexane extract; PC = positive control (50 mg/mL cisplatin). Results are expressed as mean percentages ± SEM of three independent tests, each with internal triplicate determinations (n=9). * indicates results that are significantly different to the untreated control (p<0.01).

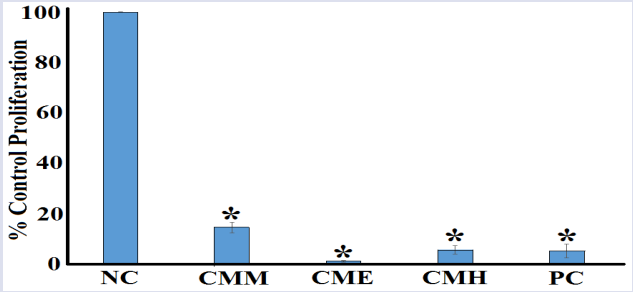

Figure 2: Anti-proliferative activity of the *C. maritima* extracts and untreated controls against HeLa cancer cells measured as percentages of the untreated control cells. NC = untreated cell growth (negative control); CMM = *C. maritima* methanolic extract; CME = *C. maritima* ethyl acetate extract; CMH = *C. maritima* hexane extract; PC = positive control (50 mg/mL cisplatin). Results are expressed as mean percentages ± SEM of three independent tests, each with internal triplicate determinations (n=9). * indicates results that are significantly different to the untreated control (p<0.01).

Table 2: The IC_{50} values (µg/mL) of CaCo2 and HeLa human cancer cell lines and the LC_{50} values (µg/mL) for *Artemia franciscana* nauplii exposed to *C. maritima* extracts and control solutions.

	Antiproliferative Activity IC_{50} (µg/mL)		Artemia nauplii LC_{50} (µg/mL)
	CaCo2	HeLa	
CMM	2261	2046	-
CME	185	468	1361
CMH	12	126	-
PC	69	85	154

Numbers indicate the mean IC_{50} or LC_{50} values of at least triplicate determinations. - indicates no significant brine shrimp mortality was evident. CMM = *C. maritima* methanolic extract; CME = *C. maritima* ethyl acetate extract; CMH = *C. maritima* hexane extract; PC = positive control (cisplatin for anti-proliferative assays; potassium dichromate for toxicity assays).

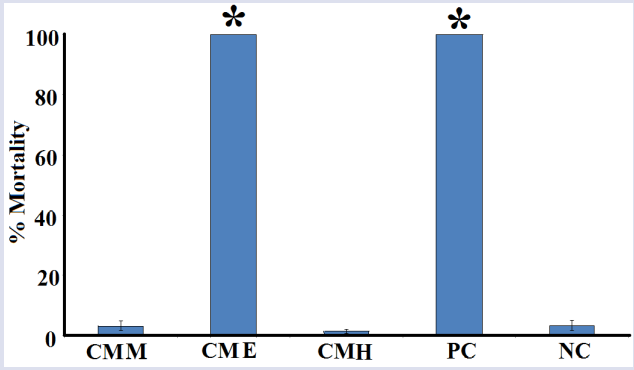

Figure 3: The lethality of the *C. maritima* extracts (2000 µg/mL) and the potassium dichromate control (1000 µg/mL) towards *Artemia franciscana* nauplii following 24 h exposure. CMM = *C. maritima* methanolic extract; CME = *C. maritima* ethyl acetate extract; CMH = *C. maritima* hexane extract; NC = negative (seawater) control; PC = positive control (1000 µg/mL potassium dichromate). Results are expressed as mean percentages ± SEM of three independent tests, each with internal triplicate determinations (n=9). * indicates results that are significantly different to the untreated control (p<0.01).

respectively) and ethyl acetate extracts (IC_{50} 185 and 468 µg/mL against CaCo2 and HeLa cells respectively) were both potent inhibitors of the proliferation of both cell lines. In contrast, the methanolic extract displayed relatively low activity (IC_{50} 2261 and 2046 µg/mL against CaCo2 and HeLa cells respectively).

Quantification of toxicity

All extracts were initially screened undiluted in the assay (Figure 3). For comparison, the reference toxin potassium dichromate (1000 µg/mL) was also tested in the bioassay. The potassium dichromate reference toxin was rapid in its onset of mortality, inducing significant nauplii death within the first 4 h of exposure and 100 % mortality within 5 h (results not shown). In contrast, the methanolic and hexane *C. maritima* extracts did not induce mortality rates significantly different from those of the untreated seawater control. However, the ethyl acetate extract induced >50 % mortality at 24 h. Therefore, methanolic and hexane *C. maritima* extracts were deemed non-toxic, whilst the ethyl acetate extract was deemed toxic (based on the screening study mortality) and its level of toxicity was further evaluated.

To further quantify the effect of toxin concentration on the induction of mortality, the *C. maritima* ethyl acetate extract was serially diluted in artificial seawater to test across a range of concentrations in the *Artemia* nauplii bioassay. Table 2 shows the LC_{50} values of the *C. maritima* extracts towards *A. franciscana*. No LC_{50} values are reported for the methanolic and hexane extracts as <50 % mortality was seen for all concentrations tested. In contrast, a 24 h LC_{50} value of 1361 µg/mL was determined for the ethyl acetate extract. As LC_{50} >1000 µg/ml towards *Artemia* nauplii for crude extracts have been defined nontoxic,[25] the ethyl acetate was also deemed to be non-toxic.

Non-targeted GC-MS headspace analysis

As the *C. maritima* hexane extract had the most potent anti-proliferative activity against both the CaCo2 and HeLa cell lines (as determined by

IC_{50}; Table 2), it was deemed the most promising extract for further phytochemical evaluation by GC-MS metabolomics profiling. The resultant gas chromatogram is presented in Figure 4. Major peaks were evident in the *C. maritima* hexane extract at approximately 13.2, 15.1, 15.6, 15.8, 15.9, 16.4, 19.1, 19.3, 20.9, 21.7, 23.6 and 24.2 min. Several smaller peaks were also evident throughout all stages of the chromatograms. In total, 137 unique mass signals were noted for the *C. maritima* hexane extract (Table 3). Putative empirical formulas and identifications were achieved for 97 of these compounds (approximately 71 %) by comparison with the database. The major components by area were: Peak 1: 2-hydroxy-1, 8-cineole (12.7 min; 1.2 % of the total area under all chromatographic peaks; Figure 5a). Peak 2: decane (13.2 min; 5.1 % of the total area under all chromatographic peaks). Peak 3: limonene (14.1 min; 2.0 % of the total area under all chromatographic peaks; Figure 5b). Peak 4: citronellal (14.6 min; 0.4 % of the total area under all chromatographic peaks; Figure 5c). Peak 5: 3, 3-dimethylhexane (15.1 min; 3.0 % of the total area under all chromatographic peaks). Peak 6: m-tolualdehyde (15.6 min; 2.5 % of the total area under all chromatographic peaks). Peak 7: isodecyl methacrylate (15.8 min; 2.2 % of the total area under all chromatographic peaks). Peak 8: 2,5-dimethyl-2-undecane (15.9 min; 2.6 % of the total area under all chromatographic peaks). Peak 9: 2,5-dimethyl-2-undecane (15.9 min; 2.6 % of the total area under all chromatographic peaks). Peak 10: nonaldehyde (16.4 min; 5.0 % of the total area under all chromatographic peaks). Peak 11: methyl salicylate (19.1 min; 3.2 % of the total area under all chromatographic peaks). Peak 12: dodecane (19.4 min; 3.3 % of the total area under all chromatographic peaks). Peak 13: 1,3-bis(1,1-dimethylethyl)-benzene (20.9 min; 10.7 % of the total area under all chromatographic peaks). Peak 14: 4,6-dimethyldodecane (21.7 min; 1.9 % of the total area under all chromatographic peaks). Peak 15: 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (23.6 min; 6.0 % of the total area under all chromatographic peaks); peak 16: propanoic acid, 2-methyl-3-hydroxy-2,2,4-trimethypentyl ester (24.2 min; 8.3 % of the total area under all chromatographic peaks).

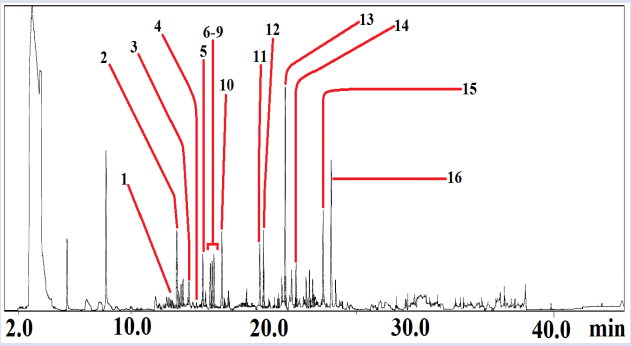

Figure 4: GC headspace total ion chromatogram of 0.5 µL injection of *C. maritima* hexane extract. The extract was dried and resuspended in methanol for analysis. The major components (by % prevalence) and some notable compounds are indicated on the chromatogram: (1) 2-hydroxy-1,8-cineole; (2) decane; (3) limonene; (4) citronellal; (5) 3,3-dimethylhexane; (6) m-tolualdehyde; (7) isodecyl methacrylate; (8) nonaldehyde; (9) 2,5-dimethyl-2-undecane; (10) nonaldehyde; (11) methyl salicylate; (12) dodecane; (13) 1,3-bis(1,1-dimethylethyl)-benzene; (14) 4,6-dimethyldodecane; (15) 2,2,4-trimethyl-1,3-pentanediol diisobutyrate; (16) propanoic acid, 2-methyl-3-hydroxy-2,2,4-trimethypentyl ester.

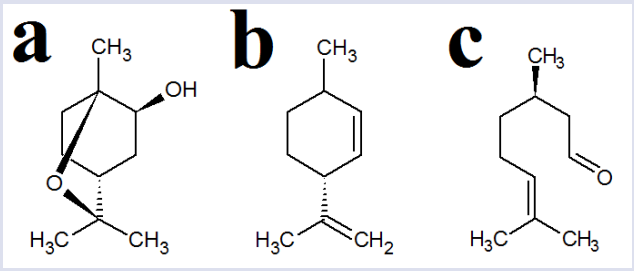

Figure 5: Chemical structures of monoterpenoid compounds detected in the *C. maritima* hexane extract by GC-MS headspace analysis: (a) 2-hydroxy-1,8-cineole; (b) limonene; (c) citronellal.

DISCUSSION

ROS, including superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$), place cells under oxidative stress and may induce damage to cellular proteins and lipids, reducing cellular survival. Cells respond to oxidative stress via both enzymatic and non-enzymatic mechanisms to minimise the effects of ROS. High intake of non-enzymatic antioxidants can scavenge ROS and has been linked with decreased incidence of some cancers.[7] However, understanding the effects of high levels of antioxidants on cancer establishment and progression has proven to be complex, with the ability of plant extracts to exert antioxidant activity dependent on multiple factors. Antioxidant phytochemicals may function as either antioxidants or pro-oxidants, depending upon their relative concentration.[26] For example, the anthraquinone aloe emodin exerts antioxidant activity at low concentrations, yet acts as a pro-oxidant at higher concentrations. In contrast, another anthraquinone (aloin) has antioxidants effect at high concentrations and pro-oxidant effects at low concentrations. Similar concentration dependent antioxidant/pro-oxidant effects have been reported for other antioxidant phytochemicals including flavonoids[27] and tannins.[28] Thus, individual phytochemicals may act as either antioxidants or pro-oxidants, dependent on their individual levels and on their relative abundances. Although an extract may have high antioxidant contents, the individual components may act either as antioxidants or as oxidants. Therefore, high antioxidant plant extracts may be effective in the treatment of cancer, as well as in its prevention at different concentrations.

The anti-proliferative efficacy of *C. maritima* extracts were examined against HeLa (cervical) and CaCo2 (colorectal) cancer cell lines. The *C. maritima* hexane extract (DPPH scavenging IC_{50} = 13.6 µg/mL) had the most potent anti-proliferative activities against the CaCo2 and HeLa cell lines, with IC_{50} values of 12 and 126 µg/mL respectively. This compares favourably with the IC_{50} values of the cisplatin control (69 and 85 µg/mL against CaCo2 and HeLa cells respectively). This is particularly impressive as the *C. maritima* hexane contains a crude mixture of phyto-compounds (only some of which would contribute to the anti-proliferative activity), whereas cisplatin is a pure compound. Purification of the anti-proliferative extract component(s) may ultimately result in a therapy with far greater efficacy than cisplatin against these cell lines. The ethyl acetate extract was also a potent inhibitor of CaCo2 and HeLa proliferation (IC_{50} values of 185 and 468 µg/mL respectively) and therefore also has potential for the development of cancer chemotherapies. The methanolic extract displayed only moderate anti-proliferative potency (IC_{50} values in the range 2000-2300 µg/mL) against both cell lines. Interestingly, the methanolic extract had substantially greater DPPH scavenging activity (IC_{50} 4.7µg/mL) than the hexane extract, indicating that the anti-proliferative activity may not correlate directly with anti-oxidant activity. Similar trends have also been reported for apple skin

Table 3: Qualitative headspace GC-MS analysis of the *C. maritima* hexane extract, elucidation of empirical formulas and putative identification (where possible) of the compounds.

Retention Time (min)	Molecular Mass	Empirical Formula	Area%	Putative Identification
10.39	151	$C_8H_9NO_2$	0.09	Methoxy-phenyl-oxime
11.72	112	$C_7H_{12}O$	1.36	(E)-2-Heptenal
12.33	128	C_9H_{20}	0.27	2,2,3,3-Tetramethylpentane
12.53	128	$C_8H_{16}O$	1.19	1-Octen-3-ol
12.74	170	$C_{10}H_{18}O_2$	1.21	2-Hydroxy-1,8-cineole
13.24	142	$C_{10}H_{22}$	5.09	Decane
13.54	142	$C_{10}H_{22}$	1.40	3,3,5-Trimethylheptane
13.67	142	$C_{10}H_{22}$	1.73	2,5,5-Trimethylheptane
13.87	120	C_9H_{12}	0.15	Ethyl-toluene
13.96	286	$C_{16}H_{30}O_4$	0.45	Oxalic acid
14.10	136	$C_{10}H_{16}$	1.99	D-Limonene
14.63	154	$C_{10}H_{18}O$	0.41	Citronellal
14.99	168	$C_{12}H_{24}$	0.27	(3Z)-9-Methyl-3-undecane
15.09	114	C_8H_{18}	2.96	3,3-Dimethylhexane
15.26	184	$C_{13}H_{28}$	0.79	Isobutylnonane
15.35	124	C_9H_{16}	0.17	2-Nonyne
15.46	130	$C_8H_{18}O$	0.28	1-Octanol
15.62	120	C_8H_8O	2.53	m-Tolualdehyde
15.75	226	$C_{14}H_{26}O_2$	2.17	Isodecyl methacrylate
15.88	182	$C_{13}H_{26}$	2.64	2,5-Dimethyl-2-undecane
16.25	184	$C_{13}H_{28}$	0.17	5-Isobutylnonane
16.44	142	$C_9H_{18}O$	4.99	n-Nonaldehyde
16.64	156	$C_{11}H_{24}$	0.43	6-Ethyl-2-methloctane
16.79	134	$C_{10}H_{14}$	0.66	1,3-Dimethylbenzene
16.92	134	$C_{10}H_{14}$	1.01	o-Cymene
17.06	126	C_9H_{18}	0.03	3,5,5-Trimethylhexene
17.25	172	$C_{11}H_{24}O$	0.05	2-Isopropyl-5-methyl-1-heptanol
18.09	286	$C_{16}H_{30}O_4$	0.31	Oxalic acid, 2-ethylhexyl isohexyl ester
18.33	184	$C_{13}H_{28}$	0.18	5-Sec-butylnonane
18.44	144	$C_8H_{16}O_2$	0.10	Octanoic acid
18.52	170	$C_{12}H_{26}$	0.03	3,8-Dimethyldecane
18.67	314	$C_{18}H_{34}O_4$	0.20	Oxalic acid, 6-ethyloct-3-yl hexyl ester
19.13	152	$C_8H_8O_3$	3.20	Methyl salicylate
19.31	150	$C_{10}H_{14}O$	0.47	Safranal
19.40	170	$C_{12}H_{26}$	3.32	n-Dodecane
19.48	156	$C_{10}H_{20}O$	0.87	Decanal
19.82	184	$C_{13}H_{28}$	0.38	4,8-Dimethylundecane
19.92	152	$C_{10}H_{16}O$	0.09	Citral
20.04	184	$C_{13}H_{28}$	0.09	4-Methyldodecane
20.16	139	$C_7H_9NO_2$	0.07	2-Ethyl-3-methyl-maleimide
20.32	374	$C_{21}H_{42}O_3S$	0.14	Sulfurous acid, cyclohexylmethyl tetradecyl ester
20.46	198	$C_{14}H_{30}$	0.60	4,6-Dimethyldodecane
20.70	198	$C_{14}H_{30}$	1.31	2,6,10-Trimethyltridecane

Continued...

Table 3: Cont'd.

Retention Time (min)	Molecular Mass	Empirical Formula	Area%	Putative Identification
20.94	190	$C_{14}H_{22}$	10.71	1,3-bis(1,1-Dimethylethyl)-benzene
21.08	154	$C_{10}H_{18}O$	0.14	2-Decenal
21.54	184	$C_{13}H_{28}$	0.26	11-Methyldodecane
21.61	168	$C_{12}H_{24}$	0.03	7-Methyl-undecene
21.71	198	$C_{14}H_{30}$	1.86	4,6-Dimethyldodecane
21.86	210	$C_{15}H_{30}$	0.28	1-Pentadecene
21.94	114	$C_{8}H_{18}$	0.45	3,3-Dimethylhexane
22.10	198	$C_{14}H_{30}$	0.14	4,6-Dimethyl-docecane
22.31	212	$C_{15}H_{32}$	0.34	2,6,11-Trimethyldodecane
22.42	200	$C_{13}H_{28}O$	1.25	Isotridecanol
22.65	200	$C_{13}H_{28}O$	1.93	n-Tridecan-1-ol
22.80	184	$C_{13}H_{28}$	0.17	5-Methyl-5-propylnonane
22.88	168	$C_{12}H_{24}$	1.32	7-Methyl-1-undecene
22.97	226	$C_{16}H_{34}$	1.02	Hexadecane
23.23	240	$C_{17}H_{36}$	0.53	Heptadecane
23.41	184	$C_{13}H_{28}$	0.15	4-Methyldodecane
23.62	286	$C_{16}H_{30}O_{4}$	5.98	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate
24.19	216	$C_{12}H_{24}O_{3}$	8.32	Propanoic acid, 2-methyl-3-hydroxy-2,2,4-trimethypentyl ester
24.96	198	$C_{14}H_{30}$	0.50	Tetradecane
25.67	264	$C_{13}H_{28}O_{3}S$	0.04	Sulfurous acid, decyl-2-propyl ester
27.83	193	$C_{13}H_{20}O$	0.06	trans-β-Ionone
28.14	282	$C_{20}H_{42}$	0.17	n-Eicosane
28.37	296	$C_{21}H_{44}$	0.73	n-Heneicosane
28.86	206	$C_{14}H_{22}O$	0.19	2,4-Di-tert-butylphenol
29.50	180	$C_{11}H_{16}O_{2}$	0.87	5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone
29.81	184	$C_{13}H_{28}$	0.04	5-Butylnonane
29.91	228	$C_{15}H_{32}O$	0.07	3,7,11-Trimethyl-1-dodecanol
30.00	352	$C_{16}H_{33}I$	0.05	n-Hexadecyl iodide
30.42	210	$C_{15}H_{30}$	0.04	Nonylcyclohexane
30.51	338	$C_{24}H_{50}$	0.12	11-Methyl-n-tricosane
30.68	256	$C_{16}H_{32}O_{2}$	0.04	Palmitic acid
30.86	380	$C_{27}H_{56}$	0.11	2-Methyl-hexacosane
31.08	296	$C_{21}H_{44}O$	0.10	8-Hexylpentadecane
31.67	368	$C_{24}H_{48}O_{2}$	0.10	Ethyl docosanoate
31.78	286	$C_{16}H_{30}O_{4}$	0.80	1-Isobutyl-4-isopropyl-3-isopropyl-2,2-dimethylsuccinate
33.00	212	$C_{15}H_{32}O$	0.17	Pentadecane
33.96	252	$C_{18}H_{36}$	0.46	n-Dodecyclohexane
33.43	356	$C_{22}H_{44}O_{3}$	0.04	Carbonic acid, decyl undecyl ester
34.10	226	$C_{16}H_{34}$	0.32	2,6,10-Trimethyltridecane
34.19	380	$C_{27}H_{56}O$	0.07	2-Methyl-n-hexacosane
34.96	208	$C_{15}H_{28}$	0.06	1,3-Dicyclohexylpropane
35.03	254	$C_{18}H_{38}$	0.02	2-Methyl-heptadecane
35.15	576	$C_{32}H_{65}I$	0.03	1-Iodo-dotriacontane

Continued...

Table 3: Cont'd.

Retention Time (min)	Molecular Mass	Empirical Formula	Area%	Putative Identification
35.47	270	$C_{17}H_{34}O_2$	0.09	Ethyl pentadecanoate
36.13	278	$C_{20}H_{38}$	0.01	Neophytadiene
36.19	268	$C_{18}H_{36}O$	0.50	6,10,14-Trimethyl-1-pentadecanone
36.48	278	$C_{16}H_{22}O_4$	0.76	Diisobutyl phthalate
36.81	312	$C_{20}H_{40}O_2$	0.02	Stearic acid, ethyl ester
37.20	256	$C_{16}H_{32}O_2$	0.43	Methyl 12-methyltetradecanoate
37.57	652	$C_{38}H_{68}O_8$	0.02	Ascorbic acid 2,6-dihexadecanoate
37.63	278	$C_{16}H_{22}O_4$	0.08	Dibutyl phthalate
37.98	284	$C_{18}H_{36}O_2$	0.72	Ethyl palmitate
38.06	184	$C_{13}H_{28}$	0.02	5-Isobutylnonane
39.77	306	$C_{20}H_{34}O_2$	0.03	Linolenic acid, ethyl ester

The % area is expressed as a % of the total area under all chromatographic peaks.

extracts against CaCo2 cells.[29] The phenolic acids and flavonoid components of the extracts may protect cells from oxidative stress by shielding lipids, proteins and DNA from oxidative damage, thereby blocking apoptosis.

GC-MS headspace analysis putatively identified a number of compounds that may contribute to the anti-proliferative activity of the *C. maritima* hexane extract. The presence of a several terpenoids was of particular interest. Indeed, the monoterpenoids 2-hydroxy-1,8-cineole (Figure 5a), limonene (Figure 5b) and citronellal (Figure 5c) were amongst the most abundant compounds identified in the extract. Additionally, the *C. maritima* hexane extract contained several other terpenoids in lower abundance. Safranal, cymene and citral were also detected in the *C. maritima* hexane extract, albeit in substantially lower relative abundance. Many monoterpenoids have anticancer properties and their anti-proliferative properties have been extensively reported. Geraniol and iosgeraniol inhibit the proliferation of murine leukaemia, hepatoma and melanomas.[30,31] Cineole, induces apoptosis in HCT116 and RKO human colon carcinoma cells by activating p38 and of caspase-3, as well as inactivating of Akt.[32] α-Pinene treatment disrupts mitochondrial membrane potential in metastatic melanoma cells, increasing their production of ROS and thereby increasing caspase-3 activity and DNA fragmentation, inducing apoptosis.[33] Linalool and its derivatives have cytostatic and cytotoxic effects in leukaemia cell lines via activation of p53 and cyclin dependent kinase inhibitors.[34] Of further note, linalool also reverses doxorubicin resistance in MCF7 AdrR multidrug resistant human breast adenocarcinoma cells and therefore potentiates the activity of doxorubicin.[35] Furthermore, many plant extracts with anticancer activity have been reported to be abundant in monoterpenoids.[17,36-38] It is therefore likely that the monoterpenoids detected in the *C. maritima* hexane extract may contribute to the anti-proliferative activity detected in our study. The sesquiterpenoid trans-β-ionone was also putatively identified in the *C. maritima* hexane extract, albeit in relatively low relative abundance. Multiple studies have also reported anticancer properties for sesquiterpenoids. Caryophyllene inhibits the cell cycle and induces apoptosis in human PC-3 prostate and MCF-7 breast cancer cell lines.[39] Zingiberene supresses N2a-NB neuroblastoma cell proliferation via the induction of apoptosis.[40] Similarly, phytol induces cellular apoptosis and inhibits proliferation in Molt 4B human leukaemia cells.[41] It is therefore possible that trans-β-ionone may have similar properties and may contribute to the anticancer properties of the *C. maritima* hexane extract.

The findings reported here also demonstrate that all *C. maritima* extracts were nontoxic towards *Artemia franciscana* nauplii. Extracts with LC_{50} values >1000 µg/mL towards *Artemia* nauplii have been defined as being nontoxic.[25] However, further studies using human cell lines are required to verify the safety of these extracts for therapeutic use. Furthermore, bioactivity driven separation studies are required to isolate the active components and determine their mechanism of action.

CONCLUSION

The results of this study demonstrate the potential of *C. maritima* extracts to block the growth of HeLa and CaCO2 cell lines. In particular, the hexane and ethyl acetate extracts were identified as particularly potent inhibitors of CaCO2 and HeLa cell proliferation. Furthermore, all extracts were nontoxic in the *Artemia* nauplii bioassay, indicating their therapeutic potential. A number of interesting compounds that may contribute to the extracts anticancer activity were identified by GC-MS. However, the anti-proliferative mechanisms of the extracts were not examined in this study. Further studies aimed at identifying the anti-proliferative mechanisms of these extracts are needed.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

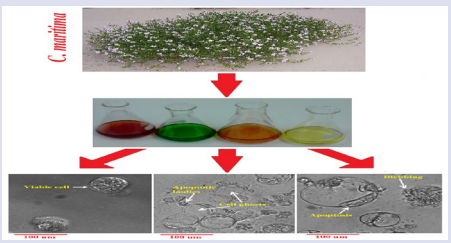
CACO2: A human colorectal cancer cell line; **DMSO:** Dimethyl sulfoxide; **DPPH:** 2,2-diphenyl-1-picrylhydrazy; **GC-MS:** Gas chromatography-mass spectrometry; **HeLa:** A human cervical cancer cell line; **IC_{50}:** The concentration required to achieve 50% effect; **LC_{50}:** The concentration required to achieve 50 % mortality; **MTS:** 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethyoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; **RPMI:** Roswell Park Memorial Institute; **ZOI:** Zone of inhibition.

REFERENCES

1. Halliwell B, Gutteridge MCJ. Free radicals in biology and medicine. 3rd ed, Oxford University Press, Oxford. 1999.
2. Hertog MG, Bueno MHB, Fehily AM, Sweetnam PM, Elwood PC, Kromhout D. Fruit and vegetable consumption and cancer mortality in the caerphilly study. Cancer Epidemiology. Biomarkers and Prevention. 1996;5(9):673-7.
3. Shalom J, Cock IE. *Terminalia ferdinandiana* Exell. fruit and leaf extracts inhibit proliferation and induce apoptosis in selected human cancer cell lines. Nutrition and Cancer. 2018;70(4):579-93. DOI: 10.1080/01635581.2018.1460680

4. Brown NS, Bicknell R. Hypoxia and oxidative stress in breast cancer. Oxidative stress: its effects on the growth, metastatic potential and response to therapy of breast cancer. Breast Cancer Research. 2001;3(5):323-7.
5. Tome ME, Baker AF, Powis G, Payne CM, Briehl MM. Catalase-overexpressing thymocytes are resistant to glucocorticoid-induced apoptosis and exhibit increased net tumor growth. Cancer Research. 2001;61(6):2766-73.
6. Holmgren A. Antioxidant function of thioredoxin and glutaredoxin systems. Antioxidants and Redox Signaling. 2000;2(4):811-20.
7. Gey KF. Cardiovascular disease and vitamins: concurrent correction of 'suboptimal' plasma antioxidant levels may, as an important part of 'optimal' nutrition, help to prevent early stages of cardiovascular disease and cancer, respectively. Bibliotheca Nutritioet Dieta. 1995;52:75-91.
8. Rice-Evans C, Miller N, Paganga G. Antioxidant properties of phenolic compounds. Trends in Plant Science. 1997;2(4):152-9.
9. Meot-Duros L, Le Floch G, Magné C. Radical scavenging, antioxidant and antimicrobial activities of halophytic species. Journal of Ethnopharmacology. 2008;116(2):258-62.
10. Guil-Guerrero JL, Giménez-Martinez JJ, Torija-Isasa ME. Nutritional composition of wild edible crucifer species. Journal of Food Biochemistry. 1999;23(3):283-94.
11. Cock IE. The phytochemistry and chemotherapeutic potential of *Tasmannia lanceolata* (*Tasmanian pepper*): A review. Pharmacognosy Communications. 2013;3(4):13-25. DOI: 10.5530/pc.2013.4.3
12. Courtney R, Sirdaarta J, Matthews B, Cock IE. Tannin components and inhibitory activity of Kakadu plum leaf extracts against microbialtriggers of autoimmune inflammatory diseases. Pharmacognosy Journal. 2015;7(1):18-31. DOI: 10.5530/pj.2015.7.2
13. Sirdaarta J, Matthews B, White A, Cock IE. GC-MS and LC-MS analysis of Kakadu plum fruit extracts displaying inhibitory activity against microbialtriggers of multiple sclerosis. Pharmacognosy Communications. 2015;5(2):100-15. DOI: 10.5530/pc.2015.2.2
14. Sirdaarta, J, Matthews B, Cock IE. Kakadu plum fruit extracts inhibit growth of the bacterial triggers of rheumatoid arthritis: Identification of stilbene and tannin components. Journal of Functional Foods. 2015;17:610-20. DOI: 10.1016/j.jff.2015.06.019
15. Sautron C, Cock IE. Antimicrobial activity and toxicity of *Syzygium australe* and *Syzygium leuhmannii* fruit extracts. Pharmacognosy Communications. 2014;4(1):53-60. DOI: 10.5530/pc.2014.1.8
16. Winnett V, Boyer H, Sirdaarta J, Cock IE. The potential of *Tasmannia lanceolata* as a natural preservative and medicinal agent: Antimicrobial activity and toxicity. Pharmacognosy Communications. 2014;4(1):42-52. DOI: 10.5530/pc.2014.1.7
17. Omer E, Elshamy A, El Gendy AN, Cai X, Sirdaarta J, White A, Cock IE. *Cakile maritima* Scop. Extracts inhibit the growth of some bacterial triggers of autoimmune diseases: GC-MS analysis of an inhibitory extract. Pharmacognosy Journal. 2016;8(4):361-74. DOI: 10.5530/pj.2016.4.9
18. McManus K, Wood A, Wright MH, Matthews B, Greene AC, Cock IE. *Terminalia ferdinandiana* Exell. extracts inhibit the growth of body odour-forming bacteria. International Journal of Cosmetic Science. 2017;39(5):500-10.
19. Winnett V, Sirdaarta J, White A, Clarke FM, Cock IE. Inhibition of *Klebsiella pneumonia* growth by selected Australian plants: natural approaches to the prevention and management of ankylosing spondylitis. Inflammopharmacology. 2017;25(2):223-35.
20. Wright MH, Arnold MSJ, Aldosary H, Sirdaarta J, Geene AC, Cock IE. Bioactive constituents of *Terminalia ferdinandiana* Exell: A pharmacognostic approach towards the prevention and treatment of yersiniosis. Pharmacognosy Communications 2016;6(3):152-63. DOI: 10.5530/pc.2016.3.5
21. Arkhipov A, Sirdaarta J, Rayan P, McDonnell PA, Cock IE. An examination of the antibacterial, antifungal, anti-Giardial and anticancer properties of *Kigelia africana* fruit extracts. Pharmacognosy Communications. 2014;4(3):62-76.
22. Lee CJ, Wright MH, Arnold MSJ, Greene AC, Cock IE. Inhibition of *Streptococcus pyogenes* growth by native Australian plants: New approaches towards the management of impetigo, pharyngitis and rheumatic heart disease. Pharmacognosy Communications. 2016;6(3):164-73. DOI: 10.5530/pc.2016.3.6
23. Hart C, Ilanko P, Sirdaarta J, Rayan P, McDonnell PA, Cock IE. *Tasmannia stipitata* as a fdunctional food/natural preservative: Antimicrobial activity and toxicity. Pharmacognosy Communications. 2014;4(4):33-47. DOI: 10.5530/pc.2014.4.4
24. Biggs I, Sirdaarta J, White A, Cock IE. GC-MS analysis of frankincense extracts which inhibit the growth of bacterial triggers of selected autoimmune diseases. Pharmacognosy Communications. 2016;6(1):10-22. DOI: 10.5530/pc.2016.1.3
25. Cock IE, Ruebhart DR. Comparison of the brine shrimp nauplii bioassay and the ToxScreen-II test for the detection of toxicity associated with Aloe vera (*Aloe barbadensis* Miller) leaf extract. Pharmacognosy Research. 2009;1(2):102-8.
26. Cock IE. Problems of reproducibility and efficacy of bioassays using crude extracts, with reference to *Aloe vera*. Pharmacognosy Communications. 2011;1(1):52-62.
27. Rahman A, Hadi SM, Parish JH. Complexes involving quercetin, DNA and Cu(II). Carcinogenesis. 1990;11(11):2001-3.
28. Singh S, Farhan AS, Ahmad A, Khan NU, Hadi SM. Oxidative DNA damage by capsaicin and dihydrocapsaicin in the presence of Cu (II). Cancer Letters. 2001;169(12):139-46.
29. Eberhardt MV, Lee CY, Liu RH. Nutrition: Antioxidant activity of fresh apples. Nature. 2000;405(6789):903-4.
30. Yu SG, Hildebrandt LA, Elson CE. Geraniol, an inhibitor of mevalonate biosynthesis, suppresses the growth of hepatomas and melanomas transplanted to rats and mice. Journal of Nutrition. 1995;125(11):2763-7.
31. Shoff SM, Grummer M, Yatvin MB, Elson CE. Concentration dependent increase of murine P388 and B16 population doubling time by the acyclic monoterpene geraniol. Cancer Research. 1991;51(1):37-42.
32. Murata S, Shiragami R, Kosugi C, Tezuka T, Yamazaki M, Hirano A, *et al.* Antitumor effect of 1, 8-cineole against colon cancer. Oncology Reports. 2013;30(6):2647-52. DOI:10.3892/or.2013.2763.
33. Matsuo AL, Figueiredo CR, Arruda DC, Pereira FV, Scutti JAB, Massaoka MH, *et al.* α-Pinene isolated from *Schinusterebinthifolius* Raddi (*Anacardiaceae*) in duces apoptosis and confers antimetastatic protection in a melanoma model. Biochemical and Biophysical Research Communications. 2011;411(2):449-54.
34. Gu Y, Ting Z, Qiu X, Zhang X, Gan X, Fang Y, *et al.* Linalool preferentially induces robust apoptosis of a variety of leukemia cells via upregulating p53 and cyclin-dependent kinase inhibitors. Toxicology. 2010;268(1):19-24.
35. Ravizza R, Gariboldi MB, Molteni R, Monti E. Linalool, a plant-derived monoterpene alcohol, reverses doxorubicin resistance in human breast adenocarcinoma cells. Oncology Reports. 2008;20(30):625-30.
36. da Silva CE, Minguzzi S, da Silva RC, Matos MF, Tofoli D, Carvalho JED, *et al.* Chemical composition and cytotoxic activity of the root essential oil from *Jatropha ribifolia* (Pohl) *Baill* (*Euphorbiaceae*). Journal of the Brazilian Chemical Society. 2015;26(2):233-8.
37. Taherkhani M, Rustaiyan A, Taherkhani T. Composition of the leaf essential oils of *Artemisia ciniformis* Krasch. et M. Pop. exPoljak, *Artemisia oliveriana* J. Gay ex Bess. in DC. And *Artemisia turanica* Krasch, three *Asteraceae* herbs growing wild in Iran. Journal of Essential Oil Bearing Plants. 2012;15(6):1006-12.
38. Tundis R, Loizzo MR, Menichini F, Bonesi M, Colica C, Menichini F. *In vitro* cytotoxic activity of extracts and isolated constituents of *Salvia leriifolia* Benth. against a panel of human cancer cell lines. Chemistry and Biodiversity. 2011;8(6):1152-62.
39. Park KR, Nam D, Yun HM, Lee SG, Jang HJ, Sethi G, *et al.* β-Caryophyllene oxide inhibits growth and induces apoptosis through the suppression of PI3K/AKT/mTOR/S6K1 pathways and ROS-mediated MAPKs activation. Cancer Letters. 2011;312(2):178-88.
40. Togar B, Turkez H, Tatar A, *et al.* Cytotoxicity and genotoxicity of zingiberene on different neuron cell lines *in vitro*. Cytotechnology. 2015;67(6):1-8.
41. Komiya T, Kyohkon M, Ohwaki S, Eto J, Katsuzaki H, Imai K, *et al.* Phytol induces programmed cell death in human lymphoid leukemia Molt 4B cells. International Journal of Molecular Medicine. 1999;4(4):377-457.

GRAPHICAL ABSTRACT



SUMMARY

- All *C. maritima* solvent extracts had strong DPPH radical scavenging activity (IC_{50} 3.4-13.6 µg/mL respectively).
- All extracts inhibited Caco2 and HeLa proliferation and this activity did not correspond with antioxidant activity.
- The hexane extract had the most potent anti-proliferative activity (IC_{50}'s of 12 and 126 µg/mL respectively against Caco2 and HeLa respectively).
- All *C. maritima* extracts were non-toxic in the the *A. franciscana* bioassay
- GC-MS profiling highlighted several compounds that may contribute to the anti-proliferative activity of the hexane extract.

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