Pharmacognosy Communications, 2025; 15(3):125-140.
https://www.phcogcommn.org

Original Article

Syzygium australe (H.L. Wendl.) B. Hyland and *Syzygium leuhmannii* (F. Muell.) L.A.S. Johnson Fruit and Leaf Extracts Inhibit Proliferation in Caco2 and HeLa Cell Lines

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ABSTRACT

Background: *Syzygium* australe (H.L.Wendl.) B.Hyland and *Syzygium leuhmannii* (F.Muell.) L.A.S.Johnson fruit and leaves have high antioxidant capacities and high levels of therapeutic phytochemicals. Despite this, the anticancer activity of *T. lanceolata* extracts has not been adequately explored. **Materials and Methods:** This study examined the antiproliferative of *S. australe* and *S. leuhmannii* fruit and leaf extracts against Caco2 colorectal and HeLa cervical carcinoma cell lines using MTS assays. The toxicity of the extracts was evaluated using *Artemia* nauplii lethality bioassays. GCMS headspace analysis was used to putatively identify extract components. **Results:** Methanolic, aqueous and ethyl acetate extracts of *S. australe* and *S. leuhmannii* fruit and leaves inhibited the proliferation of Caco2 and HeLa carcinoma cell lines. The aqueous *S. australe* fruit and *S. leuhmanii* leaf extracts were particularly potent inhibitors of Caco2 proliferation (IC_{50} = 27 µg/mL and 43 µg/mL respectively), although all aqueous extracts were potent to good inhibitors (IC_{50} values substantially <500 µg/mL) of both Caco2 and Hela proliferation. All methanolic extracts also displayed good or noteworthy antiproliferative activity, although generally with IC_{50} values substantially higher than the corresponding aqueous extracts. In contrast, the ethyl acetate extracts were generally poor inhibitors, or induced cell proliferation. **Conclusion:** The antiproliferative activity of the aqueous and methanolic *S. australe* and *S. leuhmannii* fruit and leaf extracts support their potential as cancer chemotherapies and highlight the need for further study.

Keywords: Myrtaceae, Riberry, Brush cherry, Anticancer activity, Anti-proliferative activity, Antioxidant capacity.

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Received: 27-03-2025;
Revised: 13-05-2025;
Accepted: 08-07-2025.

INTRODUCTION

Multiple recent publications have correlated the anticancer properties of several plant preparations and traditional medicines with their high antioxidant capacities. For example, plants of the genus *Terminalia* have been studied for anticancer activity due to their very high antioxidant contents and interesting phytochemistry.[1] *T. ferdinandiana* Exell. fruit extracts have potent cytotoxic effects on human HT29 colorectal adenocarcinoma and human HL60 polymyelocytic leukemia cell lines.[2] The extracts also inhibit proliferation in non-cancerous human cell lines, but did not significantly affect viability in AGS (human gastric adenocarcinoma) cells. The anti-proliferative effects of the *T. ferdinandiana* extract was associated with caspase-7, caspase-9 and poly (ADP-ribose) polymerase (PARP) activation, indicating that the extracts induced apoptosis via intrinsic pathways. A different study also reported potent anti-proliferative activity against human Caco2 colorectal, HeLa cervical, Jeg3 and JAR choriocarcinomas, as well as the human (MG63) and mouse osteosarcoma cell lines.[3] Other studies have screened human carcinoma cells against a variety of high antioxidant Asian,[4-7] Australian,[8-10] European[11] and African plants.[12] Those studies reported good anticancer activities for several plant species and correlated that activity with the antioxidant content. However, many other high antioxidant plant species are yet to be screened for anti-proliferative activity against human carcinomas.

The genus *Syzygium* (family Myrtaceae) contains approximately 500 species that are widespread in tropical and subtropical regions of Africa, Asia and Australia. Many species produce edible berries that contain high levels of antioxidants.[13,14] *Syzygium australe* (H.L. Wendl.) B.Hyland (Figure 1a-c) and *Syzygium leuhmannii* (F. Muell.) L.A.S.Johnson (Figure 1d-f) (family Myrtaceae; commonly known as brush cherry and riberry respectively) are endemic Australian trees that have been reported

DOI: 10.5530/pc.2025.3.16

to have exceptionally high antioxidant contents.[10,13] Furthermore, numerous studies have reported therapeutic properties of *Syzygium* spp., with antibacterial activity being particularly well reported.[14-18] Additionally, the anticancer properties of several *Syzygium* spp. have been reported, with the Asian species *Syzygium cumini* (L.) Skeels[19-21] and *Syzygium aromaticum* (L.) Merr. & L.M.Perry[21-23] being the most extensively studied. In contrast, the anticancer properties of Australian *Syzygium* spp. have been relatively neglected.

In addition to their high antioxidant capacities, the fruit and leaves of many *Syzygium* species have high levels of a variety of phytoconstituents with reported therapeutic properties.[24,25] In particular, many *Syzygium* spp. are rich in flavonoids and flavonoid glycosides (including quercetin and rutin), as well as terpenoids.[24] Notably, that study also listed several *Syzygium* spp. compounds with good antiproliferative activity against multiple human cancers. Despite this, the effects of Australia *Syzygium* species extracts on human cancer cells remains largely unreported. Our study was designed to screen *S. australe* and *S. leuhmannii* fruit and leaf extracts against human colorectal carcinoma Caco2 and the cervical carcinoma HeLa cell lines. These cell lines were selected as they have previously have been screened against many plant extracts and compounds, allowing for direct comparison of efficacies between studies. Furthermore, both cell lines have been extremely well characterised and their susceptibilities and resistances are well documented. Here, we report the anti-proliferative activity of *S. australe* and *S. leuhmannii* fruit and leaf and leaf extracts against these cell lines.

MATERIALS AND METHODS

Plant source and extraction

The *Syzygium australe* (H.L. Wendl.) B.Hyland and *Syzygium leuhmannii* (F. Muell.) L.A.S. Johnson fruit and leaves used in this study were obtained from the Brisbane Mt Cootha Botanical Gardens and were identified by the Gardens botanist. The fruit were dehydrated in a Sunbeam food dehydrator until a constant mass was obtained upon repeated measurements. Voucher specimens of the *S. australe* fruit (GUSAF-2017) and leaves (GUSAL-2017), as well as *S. leuhmannii* fruit (GUSLF-2017) and leaves (GUSLL-2017) are stored in the School of Environment and Science, Griffith University, Australia. Prior to extraction, the dried plant materials were ground into a coarse powder. Individual 1 g masses of the powdered *S. australe* and *S. leuhmannii* fruit and leaves were extracted separately for 24 hr at 4°C with 50 mL of methanol, deionised water or ethyl acetate. All solvents were AR grade and were purchased from Ajax Fine Chemicals, Australia. The *S. australe* and *S. leuhmannii* extracts were filtered through Whatman No. 54 filter paper and the solvent extracts were air dried at room temperature in the shade. The aqueous extracts were lyophilised at -50°C in a freeze dryer. The dried extracts were subsequently weighed and resuspended in 10 mL deionised water (containing 1% DMSO) and stored at 4°C until use.

Qualitative phytochemical studies

Qualitative phytochemical screening of the extracts for the presence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, phenolic compounds, phytoysterols, saponins, triterpenoids, and tannins was conducted by standard assays.[26]

Screen for anticancer bioactivity

Cancer cell lines

Caco2 colorectal and HeLa cervical carcinoma cells were obtained from American Type Culture Collection (ATCC, Rockville, USA). The Caco2 and HeLa cell lines were grown in Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen) supplemented with 20 mM HEPES, 10 mM sodium bicarbonate, 50 µg/mL streptomycin, 50 IU/mL penicillin, 2 mM glutamine and 10% foetal calf serum (Invitrogen, Australia). Both cell lines were grown at 37°C in a humidified 5% CO_2 environment.

Evaluation of cancer cell antiproliferative activity

The antiproliferative activity of the *S. australe and S. leuhmannii* extracts was assessed by established anti-proliferative assays.[27,28] Briefly, 70 µL of each carcinoma cell suspension (containing approximately 5000 cells), was added to the wells of a 96 well plate and 30 µL of the extract dilutions, or cell media (for the negative control) was added. The cells were incubated at 37°C, 5% CO_2 for 12 hr in a humidified atmosphere. Cisplatin (50 µg/mL) was purchased from Sigma, Australia and included on each plate as a positive control. Following the incubation, 20 µL of Cell Titre 96 Aqueous One solution (Promega) was added to each well and the plates were incubated for a further 3 hr under the same conditions. Absorbances were measured at 490 nm using a Molecular Devices, Spectra Max M3 plate reader. All tests and controls were performed three times, each with 3 internal replicates (n = 9). Antiproliferative activity was calculated and expressed as a percentage of the negative control using the formula:

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

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

Artemia franciscana nauplii lethality assays (ALA)

Toxicity of the *S. australe* and *S. leuhmannii* extracts were quantified using modified *Artemia franciscana* nauplii lethality assays.[29,30] A volume of 400 µL of the diluted plant extracts were

transferred to the wells with 400 µL of seawater containing approximately 50 *Artemia* nauplii and incubated at 25 ± 1°C under artificial light (1000 Lux). Potassium dichromate (AR grade, Chem-Supply, Australia) was prepared at 1 mg/mL and included as a reference toxin. A negative control (artificial seawater) was also included on each plate. The percentage of dead nauplii in each well was determined following 24 hr exposure and LC_{50} values with 95% confidence limits were calculated using Probit analysis. All treatments and controls were performed three times, each with internal triplicates ($n = 9$).

Calculation of therapeutic index

The therapeutic indexes (TI) of the *Syzygium* spp. extracts were calculated using the following formula and are included as a measure of the suitability of the extracts for therapeutic usage:

$$\text{Therapeutic index} = LC_{50}/MIC$$

GC-MS headspace analysis

Chromatographic separation, putative identification, and determination of the relative abundance of targeted compounds was achieved using previously optimised methods[31,32] on a Shimadzu GC-2010 Plus (USA) gas chromatography system coupled to a Shimadzu MS TQ8040 (USA) mass selective detector system. Chromatographic separation was achieved using a 5% phenyl, 95% dimethylpolysiloxane capillary column (30m x 0.25 mm internal diameter), using high grade helium as the carrier gas. The mass spectrometer was operated in the electron ionisation mode at 70 eV and mass spectral data was recorded in total ion count (TIC) mode for a 45 min period across a 45-450 m/z mass range. Structural identity was determined by comparison with mass spectral data in the National Institute of Standards and Technology (NIST) database. The calculated retention index (RIs) of each compound were then compared to RI values reported in the NIST database and compounds with RI values outside that range were considered ambiguous and are not reported herein.

Statistical analysis

The data of all experiments is expressed as the mean ± SEM of three independent experiments, each with triplicate internal replicates ($n = 9$). Statistical significance between the control and treated groups was calculated using one way ANOVA. p Values < 0.01 were considered to be statistically significant, whilst $p<0.001$ was considered to be highly significant.

RESULTS

Liquid extraction yields and qualitative phytochemical screening

Individual extractions of 1g of dried *S. australe* and *S. leuhmannii* fruit and leaf powders were extracted with solvents of varying polarity and yielded dried extracts ranging from 62 mg (*S.*

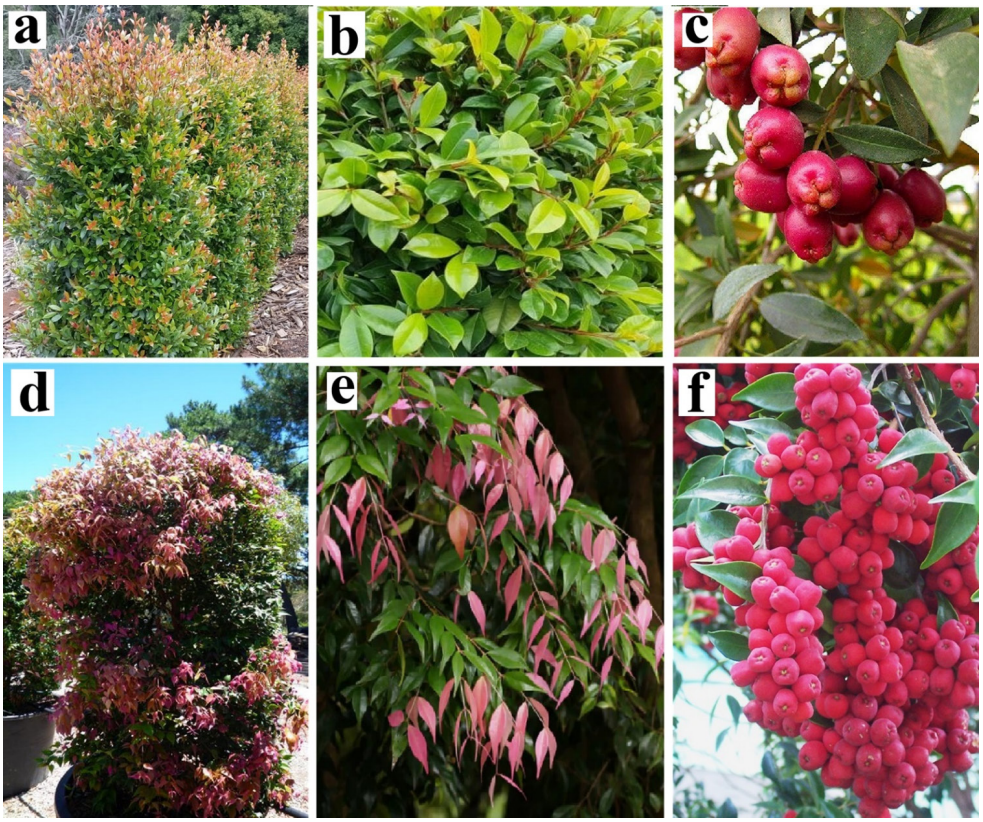

Figure 1: *Syzygium australe* (a) whole plant; (b) leaves; (c) fruit; and *Syzygium leuhmannii* (d) whole plant; (e) leaves; and (f) fruit.

leuhmannii leaf ethyl acetate extract) to 560 mg (methanolic *S. leuhmannii* fruit extract) (Table 1). Qualitative phytochemical studies showed that methanol and water also extracted a substantially wider range and greater relative abundances of phytochemicals, compared to the corresponding mid-polarity ethyl acetate extracts (Table 1). The *S. australe* fruit methanolic and aqueous extracts showed moderate to high levels of phenolics (both water soluble and insoluble phenolics) and flavonoids, as well as low levels of saponins, triterpenoids and tannins. The ethyl acetate fruit extracts generally only contained moderate levels of phenolics and low levels of flavonoids, with no other classes of phytochemicals evident. Similarly qualitative phytochemical screening of the *S. australe* leaf methanol and aqueous extracts detected moderate to high levels of phenolics (both water soluble and insoluble phenolics) and flavonoids, as well as low levels of saponins, triterpenoids and tannins The leaf ethyl acetate extract possessed similar levels of phenolic compounds, a moderate level of flavonoids and no other phytochemical classes present. Alkaloids, phytosteroids, cardiac glycosides and anthraquinones were not detected in any of the extracts.

Similar trends were observed for the *S. leuhmannii* extracts. Both the fruit and leaf methanolic and aqueous extracts contained moderate to high levels of phenolics (both water soluble and insoluble phenolics). Low levels of triterpenoids and tannins were also evident in both the fruit and leaf methanol extracts. Low levels of saponins were detected in the aqueous fruit extract, although saponins were absent in the aqueous leaf extract. Low levels of triterpenoids were detected in the aqueous leaf extract but were absent in the aqueous fruit extracts. Low to moderate levels of phenolics were also present in the fruit and leaf ethyl acetate extracts.

Antiproliferative activity against Caco2 and HeLa cell lines

The methanolic and aqueous extracts of both *S. australe* and *S. leuhmannii* extracts (fruit and leaf) strongly inhibited carcinoma cell growth, with >50% inhibition determined against both Caco2 and HeLa carcinoma cells (Figure 2). Notably, the *S. australe* methanolic and aqueous fruit and leaf extracts (with the exception of the ethyl acetate fruit and leaf extracts) significantly inhibited Caco-2 cell proliferation. Indeed, the methanolic fruit extract inhibited Caco2 proliferation by approximately 61% compared to the negative control, whilst the aqueous fruit extract inhibited Caco-2 proliferation by ~59% (Figure 2a). Similar results were noted for the *S. australe* methanolic leaf extract, which inhibited

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

	Syzygium australe						*Syzygium leuhmannii*					
	F M	F W	F E	L M	L W	L E	F M	F W	F E	L M	L W	L E
Yield (mg)	360	240	110	360	180	110	560	120	130	190	88	62
Extract concentration (mg/mL)	36	24	11	36	18	11	56	12	13	19	9	6
Total phenolics	+++	+++	++	+++	+++	++	+++	+++	++	+++	+++	++
Water soluble phenolics	+++	+++	-	+++	+++	-	+++	+++	-	+++	+++	-
Water insoluble phenolics	+++	++	++	+++	++	++	+++	++	++	+++	++	++
Flavonoids	+++	+++	+	+++	++	++	+++	+++	+	+++	++	++
Phytosterols	-	-	-	-	-	-	-	-	-	-	-	-
Saponins	-	+	-	+	+	-	+	+	-	+	+	-
Triterpenoids	+	-	-	+	-	-	+	+	-	+	+	-
Cardiac glycosides	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	+	+	-	+	-	-	+	+	-	+	+	-
Alkaloids (Mayer)	-	-	-	-	-	-	-	-	-	-	-	-
Alkaloids (Wagner)	-	-	-	-	-	-	-	-	-	-	-	-
Anthraquinones (Kumar)	-	-	-	-	-	-	-	-	-	-	-	-
Athraquinones (Aiayeoba)	-	-	-	-	-	-	-	-	-	-	-	-

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

Caco-2 proliferation by ~55%, whilst the leaf aqueous extract inhibited proliferation by 89% compared to the negative control (Figure 2b). Cisplatin (50 µg/mL), an agent commonly used in chemotherapy treatments against a variety of carcinomas, was used as a positive control in these assays. This dosage significantly inhibited Caco2 proliferation by ~86% compared to the untreated control. It is noteworthy that cisplatin is a pure compound tested at a relatively high dosage in this study, compared to the concentrations of the tested crude extracts. As crude extracts were tested, any bioactive components would be expected to be present in considerably lower concentrations in the *Syzygium* spp. extracts.

Interestingly, the *S. australe* ethyl acetate fruit and leaf extracts induced cell proliferation in the Caco2 cells. Indeed, the *S. australe* fruit ethyl acetate extract induced Caco-2 proliferation by ~55% above that of the untreated control cell proliferation. Even more dramatic, the *S. australe* leaf ethyl acetate induced ~72% Caco-2 proliferation compared to the negative control.

Similarly, *S. australe* methanolic and aqueous fruit and leaf extracts also strongly inhibited HeLa cell growth. The methanolic *S. australe* fruit and leaf extracts inhibited HeLa proliferation by 57% and 54% respectively. Additionally, the aqueous *S. australe* fruit and leaf extracts inhibited HeLa proliferation by 89% and 55% respectively. In contrast, notable differences were evident between the ethyl acetate extracts prepared using *S. australe* fruit and leaves. The fruit extract induced minor, although significant (p <0.01) inhibition of HeLa proliferation, with a decrease of ~6% compared to the negative control. In contrast, the *S. australe* ethyl acetate leaf extract was a strong inducer of HeLa proliferation, with increases of ~66% higher HeLa proliferation than the untreated control.

Screening of the *S. leuhmannii* fruit (Figure 2c) and leaf extracts (Figure 2d) yielded similar trends. As for the *S. australe* extracts, the methanolic and aqueous extracts were good inhibitors of both Caco2 and HeLa cell proliferation. Indeed, (with some notable exceptions) the *S. leuhmannii* extracts generally inhibited the growth of both Cacao2 and HeLa cells by 55-65% compared to the negative control. In contrast, the aqueous fruit extract was a substantially stronger inhibitor of Caco2 proliferation, inhibiting cell proliferation by ~95% compared to the untreated control. The methanolic leaf extract was also a good inhibitor of HeLa

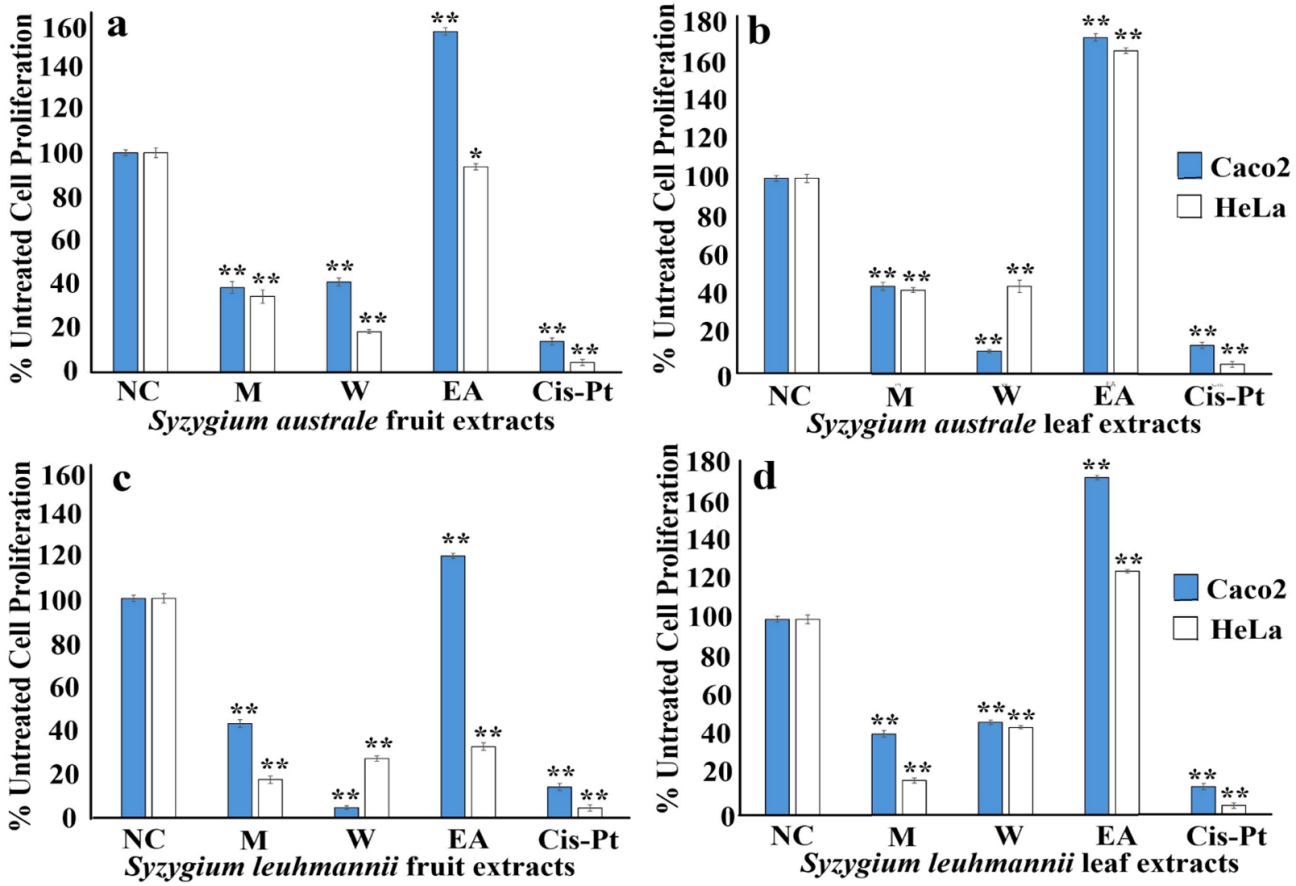

Figure 2: Anti-proliferative activity of the (a) *S. australe* fruit extract, (b) *S. australe* leaf extract, (c) *S. leuhmannii* fruit extract, and (d) *S. leuhmannii* leaf extract against Caco2 and HeLa cancer cell lines measured as percentages of the untreated control cells. NC = untreated control; M = methanolic extract; W = aqueous extract; EA = ethyl acetate extract; Cis-Pt = cisplatin control (50 µg/mL). Results are expressed as mean percentages ± SEM of three independent experiments, each with internal triplicates (n = 9). * indicates results that are significantly different to the untreated control (p <0.01), and ** indicates highly significant differences to the untreated control (p <0.001).

growth (~83% inhibition compared to the negative control). The *S. leuhmannii* ethyl acetate fruit extract was a good inhibitor of HeLa proliferation (~67% inhibition of proliferation; Figure 2c). In contrast, that extract induced proliferation in Caco2 cells (~20% increased proliferation compared to the negative control). Substantially greater increases of proliferation were noted for the *S. leuhmannii* ethyl acetate leaf extract against both carcinoma cell lines (Figure 2d). Indeed, exposure to the *S. leuhmannii* ethyl acetate leaf extract induced an increase of Caco2 proliferation of approximately 73%. Whilst less potent, this extract also induced a 25% increase in HeLa cell proliferation. In summary, the methanolic and aqueous extracts (prepared from both the fruit and leaf) were generally effective inhibitors of HeLa and Caco-2 cellular proliferation, whilst the corresponding ethyl acetate extracts generally had opposing effects.

IC_{50} Determination

The anti-proliferative potency of the *S. australe* and *S. leuhmannii* fruit and leaf extracts was further quantified by determination of their IC_{50} values (Table 2). For this study, IC_{50} values <200 µg/mL were deemed to be potent antiproliferative agents; values <500 µg/mL were classified as good antiproliferative agents, and values <1000 µg/mL were classed as noteworthy antiproliferative agents. Notably, all of the methanolic and aqueous (prepared from both *Syzygium* spp. and both plant parts) produced IC_{50} values substantially <1000 µg/mL, against both cell lines. The aqueous extracts were generally the most potent, against both Caco2 and Hela. Indeed, IC_{50} values 43 µg/mL and 128 µg/mL were recorded for the aqueous *S. leuhmannii* leaf extract against Caco2 and HeLa respectively. Similarly, IC_{50} values of 124 µg/mL and 86 µg/mL were determined for the aqueous *S. leuhmannii* fruit extract against Caco2 and HeLa cell. Similar trends were also evident for the aqueous *S. australe* fruit and leaf extracts. Indeed, IC_{50} values as low as 27 µg/mL were noted in this study (*S. australe* fruit extract against Caco2 cells).

All methanolic extracts were also noteworthy to potent inhibitors of Caco2 and HeLa cell proliferation, with the *S. australe* methanolic extracts generally being more potent that the corresponding *S. leuhmannii* extracts. However, it was not as clear which plant part produced the most effective methanolic extracts. For *S. australe*, the fruit extracts were substantially more potent inhibitors of both Caco2 and HeLa proliferation. The opposite trend was evident for the *S. leuhmannii* extracts, with the methanolic leaf extracts substantially more potent inhibitors of cell proliferation than the corresponding fruit extracts. With one notable exception (*S. australe* fruit ethyl acetate extract against HeLa; 58 µg/mL), the ethyl acetate extracts were generally ineffective inhibitors of CaCo2 and HeLa proliferation.

Quantification of toxicity

As an initial measure of toxicity, all *T. lanceolata* extracts were screened at 2000 µg/mL in the *Artemia* nauplii assay (Figure 3). Potassium dichromate (1000 µg/mL) was also included in the bioassay as a positive control. The methanolic and aqueous fruit and leaf extracts (2000 µg/mL) of both species displayed >50% mortality at 24 hr and were thus deemed to be toxic. All other extracts induced substantially <50% mortality in the ALA assay and were therefore considered to be nontoxic. To further quantify the toxicity, all extracts were tested across a range of concentrations in the ALA bioassay. LC_{50} values of 1879 and 3310 µg/mL were determined for the *S. australe* methanolic and aqueous fruit extracts respectively (Table 2). As plant extracts with LC_{50} values >1000 µg/mL have previously been defined as nontoxic in this assay,[29] these extracts were deemed to be nontoxic. In contrast, all methanolic and aqueous extracts produced from *S. australe* and *S. leuhmannii* fruit and leaves had LC_{50} values substantially <1000 µg/mL and were therefore deemed to be toxic. Notably, whilst the ALA toxicity test is generally considered to be robust, the nauplii are sensitive to changes in pH and thus low pH extracts may produce fallacious results.[29] It is likely that the high levels of ascorbic acid present in the *Syzygium* spp. extracts may contribute to their apparent toxicity, although this remains to be confirmed.[13,14] Similarly, the *S. leuhmannii* fruit ethyl acetate extract ALA tests yielded an LC_{50} of 1811 µg/mL and was therefore deemed to be nontoxicity. LC_{50} values could not be determined for any of the other ethyl acetate extracts as the mortality did not exceed 50% at any concentration tested. Therefore, those extracts were also deemed to be nontoxic.

Table 2: IC_{50} values of the *Syzygium* spp. extracts following 24 hours exposure against Caco2 and HeLa carcinoma cell lines (µg/mL), as well as LC_{50} values in *Artemia* lethality assays and the therapeutic index values (TI).

Plant Species and Part		Caco2			HeLa			LC_{50}			TI against Caco2			TI against HeLa		
		M	W	E	M	W	E	M	W	E	M	W	E	M	W	E
S. australe	Fruit	279	27	CND	134	172	58	1879	3310	CND	6.7	123	CND	14	19.2	CND
	Leaf	653	325	CND	187	283	CND	294	244	CND	0.5	0.8	CND	0.5	0.9	CND
S. leuhmannii	Fruit	791	124	CND	884	86	CND	414	478	1811	0.5	3.9	CND	0.5	5.6	CND
	Leaf	378	43	CND	165	128	CND	450	813	CND	1.2	18.9	CND	2.7	6.4	CND

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

GC-MS headspace analysis

Based on the IC_{50} values listed in Table 2, the *S. australe* fruit extracts and the *S. leuhmannii* leaf extracts were deemed to be the most potent inhibitors of proliferation. Therefore, these extracts were evaluated using GC-MS headspace analysis to putatively identify notable compounds. Numerous peaks were detected in the *S. australe* fruit extracts (Figure 4) and the *S. leuhmannii* leaf extracts (Figure 5). Multiple unique mass signals were detected in the *S. australe* extracts and their molecular weights and empirical formula were determined (Table 3). Comparison with known compound databases resulted in the putative identification of 86 compounds. Many aromatic aldehydes, ketones and aromatic benzoic compounds were identified. Several interesting compounds were identified. In particular, the monoterpenoids linalool, terpinen-4-ol, isoborneol, terpineol and methoxycitronella were present in the most potent aqueous extract, with linalool also identified in the methanolic extract. Notably, these compounds were not present or were below the detection threshold in the ethyl acetate *S. australe* fruit extract, which lacked antiproliferative activity against both cell lines. Indeed, that extract substantially induced proliferation in Caco2 cells. The presence of these compounds in the aqueous (and to a lesser extent, the methanolic extract) may contribute to the antiproliferative activity profiles reported herein, although this remains to be verified.

Similar trends were noted for the *S. leuhmannii* leaf extracts, with numerous peaks noted in the chromatograms (Figure 5). Across these extracts, 91 mass signals were putatively identified (Table 4). Of these, several peaks were identified as the monoterpenoids *trans*-linalool oxide, fenchol, β-pinone, isopinocavreol, borneol, levomenthol, terpinene-4-ol, verbenol and terpineol. As noted for the *S. australe* fruit extracts, these compounds were generally only detected in the aqueous and methanolic *S. leuhmannii* leaf extracts, and were absent in the nonactive ethyl acetate extracts. Therefore, these monoterpenoids may also contribute to the antiproliferative activity of those extracts. Notably, the sesquiterpenoids patchoulane and caryophyllene oxide were also putatively identified in some *S. leuhmannii* leaf extracts.

DISCUSSION

Plant based preparations are important targets for new drug discovery, particularly for the discovery of novel anticancer medicines. Notably, many of the current retinue of clinical anticancer drugs were originally isolated from plants, or are semi-synthetic analogues of plant-derived compounds.[33] Drugs such as paclitaxel (derived from taxol isolated from *Taxus brevifolia* Nutt.) and vincristine and vinblastine (isolated from *Catharanthus roseus* (L.) G.Don.) are perhaps the best know examples of plant-dervied anticancer drugs. Unfortunately, many cancer chemotherapeutics are inadequate for the treatment of a myriad of aggressive cancers, or are losing their efficacy against cancer cells that are acquiring resistance to their actions.[34,35] For example, pancreatic cancer often requires a concurrent high dosage of chemotherapeutics and radiotherapy to arrest

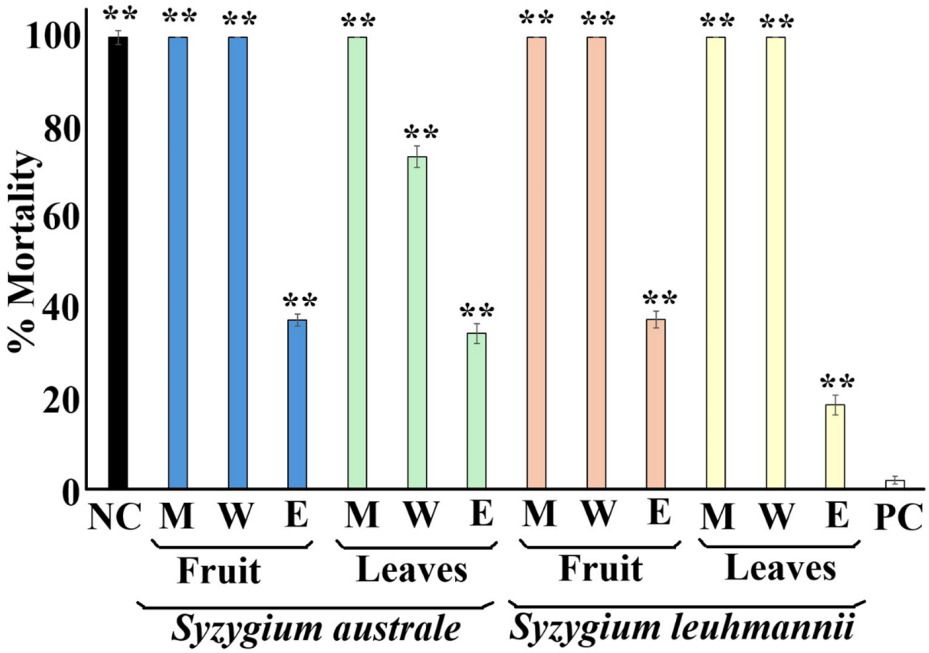

Figure 3: The lethality of the *S. australe* and *S. leuhmannii* fruit and leaf extracts (2000 µg/mL) and the potassium dichromate control (1000 µg/mL) towards *Artemia* nauplii following 24 hours exposure. NC = negative control; M = extract; W = aqueous extract; E = ethyl acetate extract; PC = positive control (1000 µg/ml potassium dichromate). Results are expressed as mean ± SEM of three independent experiments, each with internal triplicates (n =9). * indicates results that are very significantly different to the negative control (p <0.001).

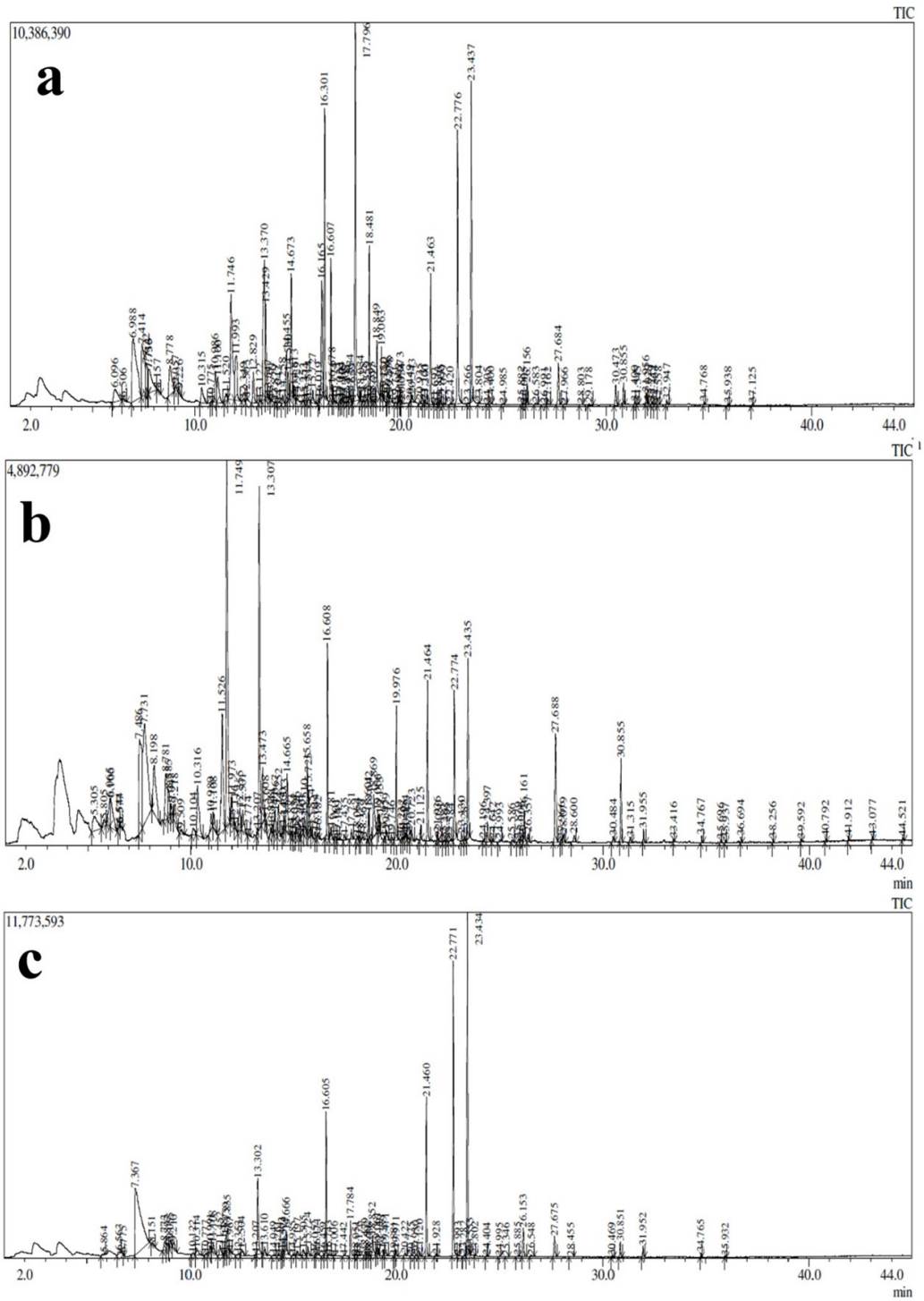

Figure 4: GC-MS headspace chromatogram of *S. australe* fruit (a) methanol, (b) aqueous, and (c) ethyl acetate extracts.

angiogenesis and metastasis. This is ultimately detrimental to the patient as these treatments are highly toxic and patients may suffer from acute chemical poisoning from the treatment. Therefore, there is a need to develop new cancer drugs to provide a continued supply of effective therapies.

Fruit and leaf extracts produced from the Australian *Syzygium* species *S. australe* and *S. leuhmannii*, were selected for antiproliferative screening based on previous reports of their exceptionally high antioxidant activities.[13,14] The extracts were screened against HeLa human cervical cancer and Caco2 colorectal cell lines to establish their anti-proliferative properties. Notably, the aqueous and methanolic extracts of both species and plant were good inhibitors of Caco2 and HeLa proliferation, whilst the corresponding ethyl acetate extracts were generally inactive, or had substantially lower potency. Our study did not determine whether the effects of the extracts were due to cytotoxic or

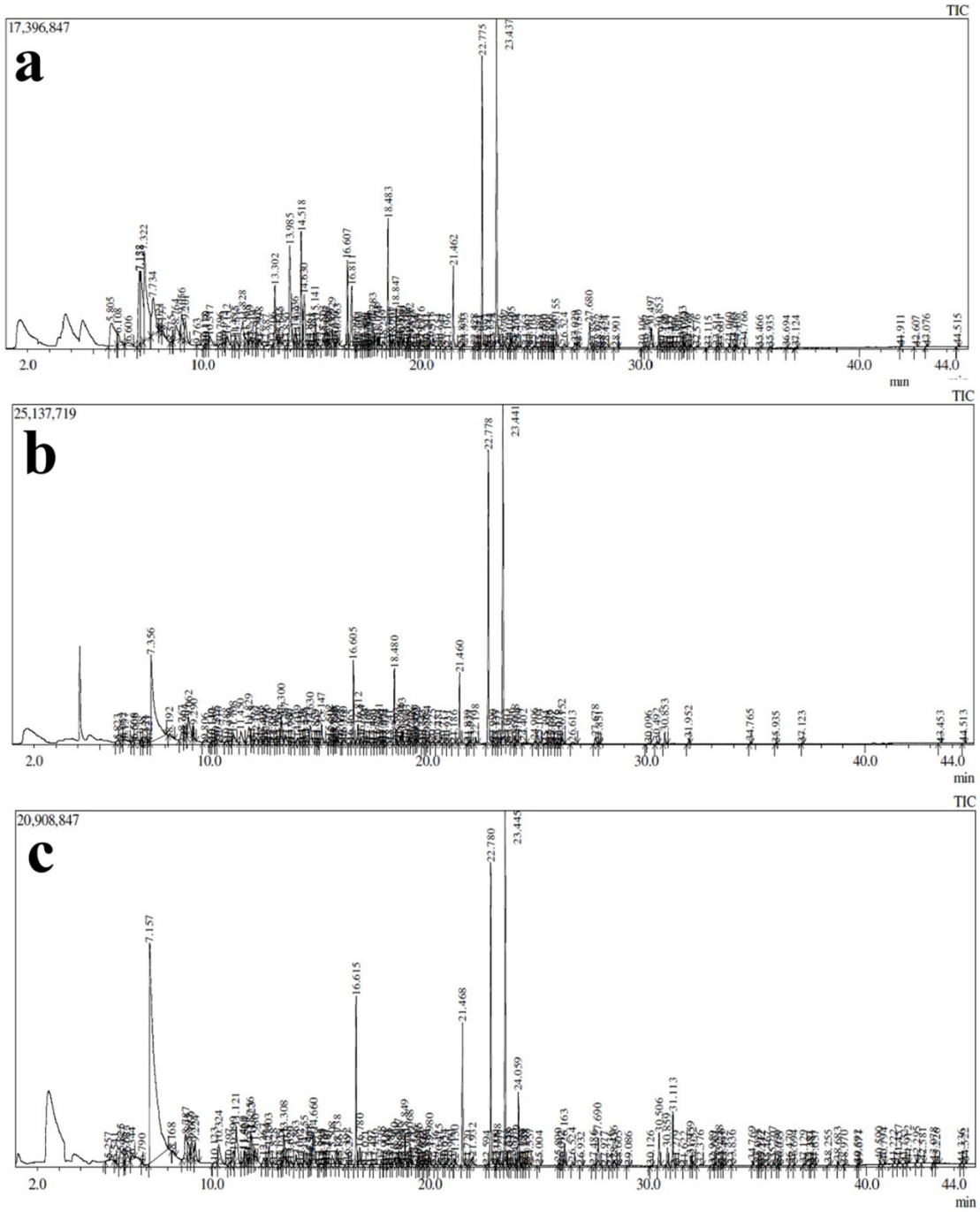

Figure 5: GC-MS headspace chromatogram of *S. leuhmannii* leaf (a) methanol, (b) aqueous, and (c) ethyl acetate extracts.

Table 3: GC-MS headspace analysis of *S. australe* fruit extracts, showing retention time, empirical formula, molecular weight determination and putative identification of extracted compounds.

Retention time (mins)	Empirical formula	Molecular Weight (Da)	Putative Identification	% Relative abundance		
				M	W	E
5.305	$C_5H_{12}O$	88	1-Pentanol	1.57		
5.805	$C_6H_{12}O$	100	2-Hexanone	0.33		
6.096	$C_6H_{12}O$	100	Hexanal	1.02		
6.106	$C_6H_{14}O$	102	2-Hexanol	1.9	1.41	

Retention time (mins)	Empirical formula	Molecular Weight (Da)	Putative Identification	% Relative abundance		
				M	W	E
6.988	$C_5H_4O_2$	96	3-Furaldehyde		7.65	
7.731	$C_6H_{12}O$	100	trans-3-Hexenol	10.09		
8.198	$C_6H_{14}O$	102	1-Hexanol	3.37	0.06	0.19
8.781	C_8H_8	104	Cyclooctatetraene	2.22	2.2	0.9
8.885	$C_6H_{10}O$	98	Cyclohexanone	1.1		0.67
9.047	$C_7H_{16}O_3$	148	Methoxyacetaldehyde diethyl acetal	0.4		0.62
9.057	$C_7H_{14}O_4$	162	2,2-Dimethoxy-1-methylethyl acetate		0.2	
9.509	C_6H_8O	96	Sorbaldehyde	0.2		
10.122	$C_8H_{18}O$	130	4-Methyl-4-heptanol			0.23
10.316	$C_6H_{16}N$	116	3-Dimethylamino-N-methylpropylamine	2.5		
10.32	$C_8H_{16}O$	128	4-Methyl-2-heptanone		0.9	0.98
10.989	$C_6H_{14}O_2$	118	5-Methoxy-1-pentano	0.47		
11.108	$C_6H_6N_4$	134	1-Aminobenzotriazole	0.55	1.4	0.79
11.52	$C_7H_{16}O$	116	1-Heptanol		0.33	0.96
11.749	$C_9H_{18}O$	142	1-Vinylheptanol	12.08	5.82	
11.973	$C_9H_{18}O$	142	4,6-Dimethyl-2-heptanone	0.79		
12.355	$C_{14}H_{26}O_3$	242	Carbonic acid, decyl prop-1-en-2-yl ester	0.81	0.11	0.18
12.505	$C_8H_{16}O$	128	Octanal		0.07	0.66
12.83	$C_{10}H_{18}O$	154	Linalool	0.07	1.09	
13.207	$C_9H_{20}O$	144	4-Methyl-4-octanol	0.09		
13.307	$C_8H_{18}O$	130	2-Ethyl-1-hexanol	8.71		5.48
13.473	$C_9H_{16}O$	140	2,2,6-Trimethylcyclohexanone	1.61		
13.608	$C_8H_{14}O$	126	3,5-Octadien-2-ol	0.21		0.61
13.37	$C_8H_{18}O$	130	Ethylhexanol		6.53	
13.43	$C_6H_{10}O_4$	146	Dimethyl butanedioate		2.38	
14.067	$C_{12}H_{26}$	170	5,6-Dimethyldecane	0.78		
14.252	$C_7H_{13}N$	111	Heptanonitril	0.78		
14.26	$C_8H_{14}O$	126	(E)-2-Octenal		0.24	0.08
14.46	$C_8H_{11}N_3$	149	1-Phenyl-3,3-dimethyltriazene	0.13	1.42	0.28
14.533	$C_8H_{16}O$	128	Cyclooctanol	0.24	0.62	0.32
14.815	$C_7H_{14}O_2$	130	Heptanoic acid	1.79	0.26	
14.883	$C_8H_{16}O$	128	2-Ethylcyclohexanol	0.13		
14.985	$C_9H_{20}O$	144	2,4-Dimethyl-1-heptanol			0.08
15.034	$C_8H_{14}O$	126	(E)-2-Octenal	0.14		
15.276	$C_{10}H_{18}O_3$	186	Carbonic acid, hexyl prop-1-en-2-yl ester	0.08		
15.658	$C_8H_{12}O$	124	1-Acetyl-2-methylcyclopentene	1.4		
15.725	$C_9H_{18}O$	142	Nonanal	0.77	0.41	0.62
15.854	$C_8H_{16}O$	128	1-Methylcycloheptanol	0.15		
16.044	$C_8H_{16}O_2$	144	2-Ethyl-hexanoic acid	0.1		0.07
16.165	$C_{10}H_{18}O$	154	exo-Fenchol		3.33	

Retention time (mins)	Empirical formula	Molecular Weight (Da)	Putative Identification	% Relative abundance		
				M	W	E
16.301	$C_6H_{10}O_5$	162	Dimethyl malate		6.41	
17.033	$C_{10}H_{16}O$	152	Alcanfor		0.05	
17.105	$C_{10}H_{18}O$	154	Linalool		0.05	
17.434	$C_{12}H_{24}$	168	(4Z)-3-Methyl-4-undecene		0.03	
17.435	$C_9H_{16}O$	140	(2Z)-2-Nonenal	0.14		
17.534	$C_{10}H_{18}O$	154	Isoborneol		0.3	2.06
17.775	$C_{10}H_{20}O$	156	1,2-Epoxydecane			
17.781	$C_{10}H_{19}N$	153	3-Pinanylamine	0.25		
17.795	$C_{10}H_{18}O$	154	Isoborneol		9.5	
18.03	$C_{10}H_{18}O$	154	Terpinen-4-ol		0.19	
18.179	$C_{11}H_{24}O$	172	2-Decanol, methyl ether	0.07		
18.481	$C_{10}H_{18}O$	154	Terpineol		3.41	0.23
18.59	$C_{11}H_{24}$	156	Undecane		0.05	0.11
19.065	$C_8H_9NO_2$	151	3,5-Dimethylnitrobenzene		1.06	0.36
19.066	$C_9H_{13}N$	135	.beta.-Methylbenzeneethanamine	0.32		
19.389	$C_{12}H2_4$	168	1-Methyl-2-pentylcyclohexane			0.29
19.395	$C_8H_{18}O_2$	146	1,2-Octanediol	0.05	0.2	
19.471	$C_{10}H_{20}O_2$	172	2-Heptyl-1,3-dioxolane			0.56
19.479	$C_{10}H_{16}O_2$	168	2-(6-Heptynyl)-1,3-dioxolane	0.32	0.21	
19.976	$C_{10}H_{12}O$	148	Benzyl ethyl ketone	2.47	0.23	
20.054	$C_{10}H_{16}O$	152	Carvotanacetone, (+)-	0.05	0.05	
20.542	$C_9H_{18}O_2$	158	Nonanoic acid	0.05	0.19	
20.723	$C_{12}H_{22}O_3$	214	Carbonic acid, nonyl vinyl ester	0.31		
20.935	$C_{10}H_{12}O_2$	164	2-Hydroxy-iso-butyrophenone		0.06	0.11
21.125	$C_{10}H_{20}O_3$	188	Butyl butoxyacetate	0.24	0.07	0.24
21.464	$C_{10}H_{20}O_2$	172	2-Heptyl-1,3-dioxolane	2.8	2.62	7.02
22.42	$C_{11}H_{22}O_2$	186	Methoxycitronella		0.04	
22.774	$C_{13}H_{26}O_2$	214	1-Methyloctyl butyrate	3.02	6.27	14.32
23.13	$C_9H_{16}O_2$	156	2(3H)-Furanone, dihydro-5-pentyl-	0.16		
23.435	$C_{12}H_{24}O_3$	216	2-Ethyl-3-hydroxyhexyl 2-methylpropanoate	3.62	6.8	16.52
23.806	$C_{12}H_{24}O_3$	216	1,3-Pentanediol, 2,2,4-trimethyl-, 1-isobutyrate	0.05		
26.357	$C_{15}H_{26}$	206	Patchoulane	0.2		
26.583	$C_{12}H_{26}O$	186	1-Dodecanol		0.08	0.13
28.079	$C_{10}H_{16}O$	152	Isoneral	0.2		
28.6	$C_{10}H_{18}O$	154	3,3,6-Trimethyl-4,5-heptadien-2-ol	0.27		
30.484	$C_{14}H_{24}O$	208	13-Tetradecen-11-yn-1-ol	0.16	0.58	0.85
32.22	$C_{14}H_{24}O$	208	13-Tetradecen-11-yn-1-ol		0.05	
33.416	$C_{16}H_{34}$	226	2,6,10-Trimethyltridecane	0.05		
35.696	$C_{15}H_{30}O_2$	242	n-Dodecyl glycidyl ether	0.05		

Table 4: GC-MS headspace analysis of *S. leuhmannii* leaf extracts, showing retention time, empirical formula, molecular weight determination and putative identification of extracted compounds.

Retention time (mins)	Empirical formula	Molecular Weight (Da)	Putative Identification	% Relative abundance		
				M	W	E
5.805	$C_6H_{12}O_2$	116	Methyl tetrahydrofurfuryl ether	4.04	0.05	
5.94	$C_6H_{10}O$	98	1-Butenyl methyl ketone			0.33
6.01	$C_6H_{14}O_2$	118	3-Methyl-2,4-pentanediol			0.85
6.157	$C_6H_{12}O$	100	n- Caproaldehyde	1.09	0.29	
6.35	$C_6H_8O_2$	112	Vinyl crotonate			0.31
6.565	$C_7H_{16}O$	116	4-Methyl-2-hexanol		0.29	
7.225	$C_5H_4O_2$	102	Furfural	17.75	0.03	
7.775	C_8H_{10}	106	Ethylbenzene	6.62	4.07	
8.16	$C_6H_{14}O$	102	Isohexanol			0.07
8.177	$C_6H_{14}O$	102	1-Hexanol	0.3		
8.77	C_8H_8	104	1,3,5,7-Cyclooctatetraene	1.82		
8.895	$C_6H_{10}O$	98	Cyclohexanone			0.85
9.06	$C_7H_{16}O_3$	148	1,1-Diethoxy-2-methoxyethane	1.62	2.87	
9.07	$C_6H_{14}O_4$	150	Tetramethoxy-ethane			0.99
9.201	$C_8H_{18}O$	130	2,3-Dimethyl-3-hexanol	1.35		0.12
9.763	$C_7H_{16}O$	116	2,3-dimethyl-1-Pentanol	0.04		
10.325	$C_8H_{16}O$	128	4-Methyl-2-heptanone	0.89	0.29	1.36
10.972	$C_8H_{18}O$	130	4-Methyl,2-heptanol	0.13	0.1	
11.12	C_7H_6O	106	Benzaldehyde	0.54	1.05	2.59
11.452	$C_7H_{16}O$	116	1-Heptanol	0.19	1.37	
11.525	$C_8H_{14}O$	126	(5E)-5-Methyl-5-hepten-3-one	0.48		0.47
11.655	$C_8H_{14}O$	126	Vinyl amyl ketone			0.06
11.755	$C_8H_{16}O$	128	Vinyl amyl carbinol			1.1
11.98	$C_9H_{18}O$	142	4,6-Dimethyl-2-heptanone			0.45
12.169	$C_8H_{16}O$	128	6-Methyl-5-hepten-2-ol	0.3	0.41	
12.365	$C_{17}H_{32}O_3$	284	Carbonic acid, prop-1-en-2-yl tridecyl ester	0.18	0.33	0.32
12.515	$C_8H_{16}O$	128	Octanal	0.03	0.04	0.23
12.598	$C_8H_{14}O$	126	6,6-Dimethyl-2-cyclohexen-1-ol	0.28	0.33	0.75
13.062	$C_8H_{18}O$	130	3-Isopropyl-1-pentanol		0.03	
13.206	$C_9H_{20}O$	144	2,4-Dimethyl-4-heptanol	0.03	0.07	0.04
13.302	$C_8H_{18}O$	130	2-Ethyl-1-hexanol	2.44	1.9	1.07
13.472	$C_9H_{12}O_2$	152	1-Phenyl-1,2-Propanediol	0.03	0.6	
13.86	$C_{13}H_{24}O_2$	212	6-Methyl-4-heptenyl 3-methylbutanoate	0.07		0.46
13.985	$C_8H_{16}O$	128	2,4-Dimethylcyclohexanol	5.7		
14.049	$C_{17}H_{30}O_2$	266	Undec-10-ynoic acid, 4-methyl-2-pentyl ester		0.47	
14.255	$C_7H_{10}O$	110	Seudenone	0.64		0.56
14.309	$C_8H_{10}O$	122	alpha.-Methyl-benzenemethanol	0.23	0.09	
14.459	C_8H_8O	120	Acetophenone	0.64	0.18	0.18

Retention time (mins)	Empirical formula	Molecular Weight (Da)	Putative Identification	% Relative abundance		
				M	W	E
14.518	$C_9H_{18}O_2$	158	Butanoic acid, 2-methylbutyl ester	3.83		
14.54	$C_8H_{16}O$	128	2-Octen-1-ol			0.09
14.988	$C_8H_{18}O$	130	1-Heptanol, 6-methyl-	0.06		0.07
15.141	$C_{10}H_{18}O_2$	170	trans-Linalool oxide (furanoid)	0.94	1.4	
15.29	$C_9H_{18}O$	142	2-Nonanone	0.02		0.01
15.726	$C_9H_{18}O$	142	Nonanal	0.11	0.08	0.47
15.85	$C_8H_{16}O$		1-Methylcycloheptanol	0.03	0.16	
15.929	$C_8H_{10}O$	122	Phenylethyl Alcohol	0.53		
16.056	$C_8H_{16}O_2$	144	2-Ethyl-hexanoic acid	0.04		
16.105	$C_{10}H_{22}O$	158	5-Methyl-5-nonanol			0.1
16.163	$C_{10}H_{18}O$	154	exo-Fenchol	0.37	0.27	
16.68	$C_9H_{14}O$	138	beta.-Pinone	2.18		0.66
16.811	$C_{10}H_{16}O$	152	Isopinocarveol	2.91	1.31	
17.361	$C_9H_{12}O$	136	Methyl m-tolylcarbinol	0.02		
17.783	$C_{10}H_{18}O$	154	endo-Borneol	0.63	0.4	
17.908	$C_{10}H_{16}O$	152	Isocamphopinone	0.42		
17.957	$C_{10}H_{20}O$	156	Levomenthol		0.07	
18.034	$C_{10}H_{18}O$	154	Terpinen-4-ol	0.23	0.1	
18.249	$C_{10}H_{16}O$	152	cis-Verbenol	0.16		
18.26	$C_{10}H_{22}O$	158	1-Decanol			0.29
18.483	$C_{10}H_{18}O$	154	L-.alpha.-Terpineol	3.88	4.21	
18.52	$C_{10}H_{20}O_2$	172	Ethyl caprylate			0.3
18.601	$C_{12}H_{26}$	170	Dodecane	0.16	0.1	0.14
18.746	$C_9H_{16}O$	140	2-Methylene-cyclohexaneethanol	0.25	0.39	
18.935	$C_{12}H_{22}O_3$	214	Carbonic acid, nonyl vinyl ester	0.11		0.02
19.07	$C_9H_{10}O$	134	Hydratropaldehyde	0.29		
19.152	$C_{10}H_{18}O_2$	170	4,6-Dimethyloctane-3,5-dione	0.21	0.27	0.08
19.35	$C_{12}H_{24}$	168	1-Methyl-2-pentylcyclohexane	0.26	0.19	0.11
20.69	$C_{10}H_{22}O$	158	2-Propyl-1-heptanol	0.05		0.04
20.944	$C_{10}H_{12}O_2$	164	2-Hydroxy-iso-butyrophenone	0.03		
21.08	$C_{10}H_{20}O_3$	188	Butyl butoxyacetate			0.15
21.462	$C_{10}H_{20}O_2$	172	2-Heptyl-1,3-dioxolane	2.58	3.59	
22.775	$C_{13}H_{26}O_2$	214	1-Methyloctyl butyrate	8.18	16.87	8.6
22.993	$C_{11}H_{22}O_2$	186	Heptyl butyrate	0.02	0.04	
23.36	$C_{12}H_{24}O_3$	216	Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester	10.08	19.14	9.78
23.806	$C_{12}H_{24}O_3$	216	2-Ethyl-3-hydroxyhexyl 2-methylpropanoate	0.2	0.09	
23.895	$C_{14}H_{28}$	196	(Z)-7-Tetradecene			0.15
24.018	$C_{15}H_{26}O_2$	238	Decenyltiglate, 2E-		0.32	
24.06	$C_{12}H_{24}O_2$	200	Capric acid, ethyl ester	0.09		1.99
24.105	$C_{15}H_{26}$	206	Patchoulane	0.31		0.03

Retention time (mins)	Empirical formula	Molecular Weight (Da)	Putative Identification	% Relative abundance		
				M	W	E
26.524	$C_{13}H_{28}O$	200	n-Tridecanol	0.13		
26.613	$C_{12}H_{22}O$	182	(E)-2-Dodecenal		0.23	
30.492	$C_{15}H_{24}O$	220	Caryophyllene oxide	0.73	0.18	1.18
30.76	$C_{10}H_{16}O$	152	Chrysanthenol			
30.855	$C_{16}H_{30}O_4$	286	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	0.57	0.76	0.56
30.99	$C_{14}H_{28}O_2$	228	Dodecanoic acid, ethyl ester	0.02		1.69
31.28	$C_{17}H_{32}O_3$	284	Carbonic acid, tetradecyl vinyl ester	0.06		0.03
32.024	$C_{15}H_{24}O$	220	trans-Z-.alpha.-Bisabolene epoxide	0.09		
35.025	$C_{15}H_{30}O_2$	242	Tridecanoic acid ethyl ester			0.05
36.43	$C_{17}H_{36}$	240	Heptadecane	0.04		0.04
38.64	$C_{17}H_{34}O_3$	286	Carbonic acid, 2-ethylhexyl octyl ester			0.08
41.415	$C_{19}H_{41}$	269	Nonadecane	0.02		0.24

cytostatic mechanisms and further work is required to understand how these extracts work. In particular, an examination of cell morphology may be useful in determining whether the extracts are inducing apoptosis or necrosis. Indeed, similar methods have previously been useful in demonstrating apoptosis induction in response to other plant extracts.[3,36] Additionally, those studies also examined the effects of plant extracts on caspase 3 levels to further understand the mechanism of apoptotic induction. Examining the effects of the *S. australe* and *S. leuhmannii* fruit and leaf extracts on caspase 3 (and other caspases) is warranted and would provide a deeper understanding of the effects of these extracts. Furthermore, the effects of the extracts on cell cycle progression should also be examined. As extracts are complex mixtures, it is possible that they may have both cytotoxic and cytostatic effects and this should be tested.

Whilst our study did not determine the compounds responsible for the antiproliferative effects of the *S. australe* and *S. leuhmannii* fruit and leaf extracts, GCMS headspace analysis was used to putatively identify extract components. A number of compounds were highlighted due to their potential to contribute to the antiproliferative activity of the extracts. Several monoterpenoids (in both the *S. australe* and *S. leuhmannii* extracts) and sesquiterpenoids (in the *S. leuhmannii* extracts only) were highlighted as possible contributors to this antiproliferative activity. Of particular note, the monoterpenoids linalool and terpineol were detected in relatively high abundance in the methanolic and aqueous extracts produced from the fruit and leaves of both *Syzygium* species. Multiple monoterpenoids, including linalool and terpineol, have been reported to induce apoptosis in multiple cancer cell lines.[37] Furthermore, the apoptotic induction was linked to oxidative stress. Notably, several monoterpenoids have been shown to suppress cell cycle progression.[37] It is therefore possible that these compounds function via several mechanisms.

Additionally, the sesquiterpenoids caryophyllene oxide and patchoulane were also putatively identified in the methanolic and aqueous *S. leuhmannii* extracts. Interestingly, caryophyllene oxide inhibits proliferation and induces apoptosis in human PC-3 prostate and MCF-7 breast cancer cells.[38] That study determined that caryophyllene oxide's anticancer effects are mediated by 2 mechanisms: an inhibition of PI3K/AKT/mTOR/S6K1 signalling cascade, and by activating cellular ERK, JNK and p38 MAPK. Caryophyllene oxide exposure induces increased levels of mitochondrial ROS, loss of mitochondrial membrane potential, release of cytochrome c, activation of caspase-3 and cleavage of PARP. Furthermore, caryophyllene oxide down-regulates the expression of cyclin D1, bcl-2, bcl-xL, survivin, IAP-1 and IAP-2, whilst stimulating the expression of p53 and p21. Caryophyllene oxide can also potentiate the apoptotic effects of other sesquiterpenoids, including humulene and isocaryophyllene.[39]

CONCLUSION

The potent antiproliferative activities described herein demonstrate the potential of the aqueous methanolic *S. australe* and *S. leuhmannii* fruit and leaf extracts to inhibit the proliferation of Caco2 and HeLa carcinoma cell lines. Furthermore, all of the *Syzygium* spp. extracts were nontoxic, providing further support for their potential in the prevention and treatment of some cancers. Further elucidation of the antiproliferative mechanism(s) is required to evaluate the potential of the extract components as cancer chemotherapeutics.

ACKNOWLEDGEMENT

Financial support for this work was provided by the Centre for Planetary Health and Food Security, Griffith University.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

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

AUTHORS CONTRIBUTIONS

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

DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon request.

REFERENCES

1. Cock IE. The medicinal properties and phytochemistry of plants of the genus *Terminalia* (Combretaceae). Inflammopharmacol. 2015; 23: 203-29. DOI: 10.1007/s10787-015-0246-z
2. Tan AC, Konczak I, Ramzan I, *et al.* Potential antioxidant, antiinflammatory, and proapoptotic anticancer activities of Kakadu plum and Illawarra plum polyphenolic fractions. Nutrit Cancer. 2011; 63: 1074-84.
3. Shalom J, Cock IE. *Terminalia ferdinandiana* Exell. fruit and leaf extracts inhibit proliferation and induce apoptosis in selected human cancer cell lines. Nutrit Cancer. 2018; 70(4): 579-93.
4. Kaur S, Michael H, Arora S, *et al.* The *in vitro* cytotoxic and apoptotic activity of Triphala: An Indian herbal drug. J Ethnopharmacol. 2005; 97: 15-20.
5. Shi Y, Sahu RP, Srivastava SK. Triphala inhibits both *in vitro* and *in vivo* xenograft growth of pancreatic tumor cells by inducing apoptosis. BMC Cancer, 2008; 8: 294.
6. Sandhya T, Mishra KP. Cytotoxic response of breast cancer cell lines, MCF-7 and T 47 D to triphala and its modification by antioxidants. Cancer Lett, 2006; 238: 304-13.
7. Pinmai K, Chunlaratthanabhorn S, Ngamkitidechakul C, *et al.* Synergistic growth inhibitory effects of *Phyllanthus emblica* and *Terminalia bellerica* extracts with conventional cytotoxic agents: Doxorubicin and cisplatin against human hepatocellular carcinoma and lung cancer cells. World J Gastroenterol, 2008; 14: 1491-7.
8. Courtney R, Sirdaarta J, White A, *et al.* Inhibition of Caco-2 and HeLa proliferation by *Terminalia carpentariae* C.T.White and *Terminalia grandiflora* Benth. extracts: Identification of triterpenoid components. Pharmacog J, 2017; 9: 175-84. DOI: 10.5530/pj.2017.2.29
9. Sirdaarta J, Maen A, Rayan P, *et al.* High performance liquid chromatography-mass spectrometry analysis of high antioxidant Australian fruits with antiproliferative activity against cancer cells. Pharmacog Mag, 2016; 12: S181-94.
10. Jamieson N, Sirdaarta J, Cock IE. The anti-proliferative properties of Australian plants with high antioxidant capacities against cancer cell lines. Pharmacog Commun, 2014; 4: 71-82. DOI: 10.5530/pc.2014.4.8
11. Omer E, Elshamy A, Taher R, et al. *Cakile maritima* Scop. Extracts inhibit Caco2 and HeLa human carcinoma cell growth: GC-MS analysis of an anti-proliferative extract. Pharmacog J, 2019; 11(2): 258-66.
12. Gu B, Shalom J, Cock IE. Anti-proliferative properties of *Terminalia sericea* Burch. Ex DC leaf extracts against Caco2 and HeLa cancer cell lines. Pharmacog J, 2018; 10(3): 408-15.
13. Netzel M, Netzel G, Tian Q, *et al*, Native Australian fruits - a novel source of antioxidants for food. Innovative Food Sci Emerg Technol, 2007; 8: 339-46.
14. Murhekar S, Wright MH, Greene AC, *et al.* Inhibition of *Shewanella* spp. growth by *Syzygium australe* and *Syzygium luehmannii* extracts: Natural methods for the prevention of fish spoilage. Internat J Food Sci Technol, 2017; 54(10): 3314-26.
15. Woods A, McManus K, Wright MH, Greene AC, Cock IE. Growth inhibitory activity of selected Australian *Syzygium* species against malodour forming bacteria. Pharmacog Commun, 2017; 7(3): 129-36.
16. Wright MH, Lee CJ, Pollock CE, *et al.* Growth inhibitory activity of selected high antioxidant Australian *Syzygium* species against the food poisoning and tissue necrotic pathogen *Clostridium perfringens*. Pharmacog Commun, 2016; 6(2): 93-9.
17. Cock IE, van Vuuren SF. The potential of selected some South African plants with anti-*Klebsiella* activity for the treatment and prevention of ankylosing spondylitis. Inflammopharmacol, 2015; 23(1): 21-35.
18. Winnett V, Sirdaarta J, White A, *et al.* Inhibition of *Klebsiella pneumoniae* growth by selected Australian plants: natural approaches for the prevention and management of ankylosing spondylitis. Inflammopharmacol, 2017; 25(2): 223-35. DOI: 10.1007/s10787-017-0328-1
19. Li L, Mangali S, Kour N, et al. *Syzygium cumini* (jamun) fruit-extracted phytochemicals exert anti-proliferative effect on ovarian cancer cells. J Cancer Res Therapeut, 2021; 17(6): 1547-51.
20. Khodavirdipour A, Zarean R, Safaralizadeh R. Evaluation of the anti-cancer effect of *Syzygium cumini* ethanolic extract on HT-29 colorectal cell line. J Gastrointest Cancer, 2021; 52: 575-81.
21. Khasanah U, Adiningsih OR, Anggraeni ED, *et al.* Phytochemical screening, total phenolic content and cytotoxic activity of seed, leaves, and pulp from *Syzygium cumini* against breast cancer cell culture 4T1. Res J Pharmacog Phytochem, 2022; 14(3): 145-9.
22. Abadi AV, Karimi E, Oskoueian E, *et al.* Chemical investigation and screening of anti-cancer potential of *Syzygium aromaticum* L. bud (clove) essential oil nanoemulsion. 3 Biotech, 2022; 12(2): 49.
23. Valizadeh A, Khaleghi AA, Alipanah H, *et al.* Anticarcinogenic effect of chitosan nanoparticles containing *Syzygium aromaticum* essential oil or eugenol toward breast and skin cancer cell lines. BioNanoScience, 2021; 11(3): 678-86.
24. Cock, IE, Cheesman MJ. The potential of plants of the genus *Syzygium* (Myrtaceae) for the prevention and treatment of arthritic and autoimmune diseases. In Bioactive Food as Dietary Interventions for Arthritis and related Inflammatory Disease. 2nd edition, 2019,
25. Cock IE, Cheesman MJ. Plants of the genus *Syzygium* (Myrtaceae): A review on ethnobotany, medicinal properties and phytochemistry. In Bioactive Compounds of Medicinal Plants, 2018,
26. Arkhipov A, Sirdaarta J, Rayan P, *et al.* An examination of the antibacterial, antifungal, anti-Giardial and anticancer properties of *Kigelia africana* fruit extracts. Pharmacog Commun, 2014; 4: 62-76. DOI: 10.5530/pc.2014.3.7
27. Omer E, Elshamy A, Taher R, et al. *Cakile maritima* Scop. extracts inhibit Caco2 and HeLa human carcinoma cell growth: GC-MS analysis of an anti-proliferative extract. Pharmacog J, 2019; 11(2): 258-66.
28. Gu B, Shalom J, Cock IE. Anti-proliferative properties of *Terminalia sericea* Burch. Ex DC leaf extracts against Caco2 and HeLa cancer cell lines. Pharmacog J, 2018; 10(3): 408-15.
29. Ruebhart DR, Wickramasinghe W, Cock IE. Protective efficacy of the antioxidants vitamin E and Trolox against *Microcystis aeruginosa* and microcystin-LR in *Artemia franciscana* nauplii. J Tox Environ Health, Part A. 2009; 72(24): 1567-75.
30. Ilanko P, McDonnell PA, van Vuuren S, *et al.* Interactive antibacterial profile of *Moringa oleifera* Lam. extracts and conventional antibiotics against bacterial triggers of some autoimmune inflammatory diseases. S Afr J Bot, 2019; 124: 420-35.
31. Grimsey L, Van Vuuren SF, Wright MH, *et al.* Selected South African *Combretum* spp. extracts inhibit methicillin-resistant *Staphylococcus aureus* and ESBL strains of *Escherichia coli* and *Klebsiella pneumoniae*. S Afr J Bot, 2024; 165: 49-58.
32. Nel AL, Murhekar S, Matthews B, *et al.* The interactive antimicrobial activity of *Terminalia sericea* Burch ex DC leaf extracts and conventional antibiotics against bacterial triggers of selected autoimmune inflammatory diseases. SAfr J Bot, 2020; 133: 17-29
33. Harvey AL. Natural products in drug discovery. Drug Discov Today 2008; 13: 894-901.
34. Neophytou CM, Trougakos IP, Erin N, *et al.* Apoptosis deregulation and the development of cancer multi-drug resistance. Cancers, 2021; 13(17): 4363.
35. Abdelmaksoud NM, Abulsoud AI, Doghish AS, *et al.* From resistance to resilience: Uncovering chemotherapeutic resistance mechanisms;insights from established models. Biochim Biophys Acta Rev Cancer, 2023: 188993.
36. Shalom J, Cock IE. *Tasmannia lanceolata* (Poir.) AC Sm. berry and leaf extracts inhibit proliferation and induce apoptosis in selected human carcinoma cell lines. Pharmacog Commun, 2023; 13(3): 126-40.

37. Silva BI, Nascimento EA, Silva CJ, *et al.* Anticancer activity of monoterpenes: A systematic review. Molec Biol Rep. 2021; 48: 5775-85.
38. Park KR, Nam D, Yun HM, *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 Lett, 2011; 312(21): 178-88.
39. Legault J, Pichette A. Potentiating effect of β-caryophyllene on anticancer activity of α-humulene, isocaryophyllene and paclitaxel. J Pharm Pharmacol, 2007; 59(12): 1643-7.

Cite this article: Shalom J, Cock ID. *Syzygium australe* (H.L. Wendl.) B. Hyland and *Syzygium leuhmannii* (F. Muell.) L.A.S. Johnson Fruit and Leaf Extracts Inhibit Proliferation in Caco2 and HeLa Cell Lines. Pharmacognosy Communications. 2025;15(3):125-40.