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Cytotoxicity, Anticancer Potentials and Anticancer Phytochemicals Present in *Viscum continuum* E. Mey. Ex *Sprague* Extracts

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Background: Cancer describes a vast category of diseases our body suffers from. The total global oncology spending was USD186bn as of 2021 and was estimated to rise to USD208.9bn in 2022. The adverse effects of managing cancer with orthodox medications includes death among other dire consequences. Several *Viscum* species including *Viscum album* L, *Viscum angulatum*, and *Viscum articulatum* Burm f. from other countries have been reported for their anticancer potentials.

Methods: Four ($n = 4$) mistletoe extracts namely *n*-hexane, dichloromethane, acetone, and methanol were tested for their anti-cancer potentials against Vero (African green monkey, kidney, non-cancerous), BJ-5ta (Human skin fibroblast), A549 (Human non-small cell lung carcinoma), Michigan Cancer Foundation (MCF7) human breast cancer cell lines. The percentage cancer cells survival rate and selectivity index for each extract were calculated against positive controls (untreated cells) and blank dimethyl sulfoxide (DMSO) solutions, and the compounds with anticancer potentials were identified to be present in the extracts using gas chromatography-mass spectrometry analysis.

Results: All four extracts showed concentration dependent anti-cancer activity as measured from the percentage average cell viability or cytotoxicity. The cytotoxicity to the lung (A549) was recorded as 1.25, 10, 0.5, 1.25 mg/mL for the hexane, dichloromethane, acetone and methanol extracts respectively. Those for the breast (MCF7) cancer cell lines appeared as 1.25, 0.5, 0.5 and 10 mg/mL for the hexane, dichloromethane, acetone and methanol extracts in that order. In terms of the selectivity index (SI), that is, which extracts is cytotoxic to a specific cancer cell line, all the extracts were highly selective ($SI > 2$). However, the methanol extract was selectively more toxic to the lung cancer cell (A549) with SI of 6.08 and Half maximal Inhibitory Concentration (IC_{50}) value of 0.251 ± 3.96 . The other three extracts were highly selective against breast cancer cell (MCF7) with SI of 5.90 ($IC_{50} = 0.38$), 9.06 ($IC_{50} = 0.04$) and 8.15 ($IC_{50} = 0.02$) for *n*-hexane, dichloromethane (DCM), and acetone extracts, respectively.

Conclusions: Eucalyptol, 9(E),11(E)-conjugated linoleic acid, ester and 9, 12, 15-octadecatrienoic acid detected in South African mistletoe by gas chromatography-mass spectrometry (GC-MS) analysis is proposed to be responsible for the anticancer potentials of the extracts.

Keywords: mistletoe; extracts; cancer cells; cytotoxicity; selectivity index

Introduction

There is an increase in reliance on medicinal plants for treatment and management of different illnesses and diseases across the globe. These plants are explored for the treatment of hereditary, communicable, and non-communicable diseases. One area where this interest can be seen is in the management of cancer because cancer is a world leader in the cause of death worldwide from the world health organization data [1]. Diseases that the body without restriction condition may be described as neoplasm, malignant tumors or cancer [1]. Cancer metastasis involves the rapid creation of abnormal cells that grow beyond their usual boundaries, metastases to adjoining body parts, organs and primarily cause death [2]. The WHO fur-

ther reported that the most common of cancers were breast, lung, colon, rectum and prostate cancers in that order [1]. Managing these cancers has and continue to be very costly with reports suggesting that the global oncology spending stood at USD186bn as of 2021 and was estimated to rise to USD208.9bn in 2022 [3,4], yet this figure is geometrically increasing. Current treatment or management usually includes radiotherapy and/or chemotherapy and/or surgery, hormone therapies, targeted cancer drugs and bisphosphonates [5,6].

Complementary and alternative medicines and in particular the use of medicinal plants offer affordable alternatives that is currently preferred in the management of cancer [7]. From the plethora of medicinal plants at mankind's disposal for used across the globe in the treatment and manage-

Table 1. Seeding protocol of the four cell lines used the study.

No.	Cell line	Cell description	Passage No.	Seeding density (Viable cells/well)
1	Vero	African green monkey, kidney, non-cancerous	39–42	5000
2	BJ-5ta	Human skin fibroblast, hTERT immortalized, non-cancerous	11–13	16,000
3	A549	Human non-small cell lung carcinoma	109–112	4000
4	MCF7	Human breast adenocarcinoma	22–25	8000

MCF7, Michigan Cancer Foundation; hTERT, Human Telomerase Reverse Transcriptase.

ment of cancer are the different species of *Viscum*. A number of studies have been conducted on these species from different parts of the world and established that most of the *Viscum* species demonstrate anticancer potential [8,9]. Although undocumented, modern herbalist in South Africa are using mistletoe tree traditionally in the treatment and management of cancer. South African *Viscum* is amongst the best medicinally important plants that is readily utilized for post-stroke management. The could grow up to 2 m long on tree trunks or their branches, has been reported by us to be a great source of antioxidants [10]. Given the major role which oxidants and antioxidants play in signal transduction and cell proliferation this plant could be a major source of anticancer agent. In as much as there are reported anticancer potentials of *Viscum album* L, *Viscum angulatum*, *Viscum articulatum* Burm f. and another type of mistletoe *Loranthus ferrugineus* [8,9,11–13], such information is not available for *Viscum continuum* E.mey. Ex *Sprague* of the South African ecotype. Only undocumented and word of mouth knowledge is available on its uses in the traditional management of malignant tumors or related cancer. Literature suggests that amongst the common groups of phytochemicals, alkaloids exhibit anticancer effect [14,15]. Several alkaloids isolated from medicinal plants including mistletoe trees have been shown to exhibit antimetastatic and antiproliferation both *in vivo* and *in vitro* on numerous cancers [14,16]. A variety of alkaloids containing oral and topical preparations made from European mistletoe (*Viscum album* L.) were used as folk remedies in ancient Greek and Celtic medicine [17]. A popular example of such mistletoe preparations is 'Iscar', a precursor of today's commercial Iscador or brand preparations that contains extracts of European mistletoe [18]. As investigated and reported the South African *Viscum continuum* E. meyer. Ex *Sprague* extracts contain alkaloids [10]. This study reports on the cytotoxicity and the anticancer potentials of four extracts of *Viscum continuum* E. meyer. Ex *Sprague* against selected breast, lung, skin, cancer cell lines that were selected because they are amongst the most common cancers that South African are diagnosed of according Finestone and Wishnia [19].

Materials and Methods

Plant Collection, Processing and Extraction

Mistletoe tree samples was harvested at on the twenty-second of July, 2019 in Molopo village South Africa Mma-

batho (25.8378° S, 25.5948° E). The plant materia was pinpointed prior by a local medicinal plant farmer and harvester. The entire plant was further identified by a personnel in the office of South African National Biodiversity Institute (SANBI) as *Viscum continuum* E. meyer. Ex *Sprague* and coded with (MTT01) receipt specimen deposited in mini-herbarium of School of Pharmacy, Sefako Makgatho Health Sciences University. After washing the plant material with tap water, it was dried at room temperature, grounded to fine powder using Polymix Laboratory Dry Mill Drive Unit (Polymix™ PX-MFC 90 D, Kinematica AG, Luzern, Switzerland) at 3500 revolutions per minute. The pooled ground powder was then sieved to enhanced uniform particle size and amassed in containers prior to further processing. All the solvents (AR grade) used in the extraction of the powdered plant material was supplied by Rochelle & Lab Equipment Cc (Johannesburg, South Africa). The 1.05 kg of the powder sample was extracted using 2.5 L of each solvent in a NUVE extraction bath (ST15, Akyurt, Turkey) at 100 Revolution per minute in a serial exhaustive cold maceration procedure in a 24 hours cycle for each run. The combined filtrate was evaporated to dryness with a Stuart rotary evaporator (Re400, Cole-Parmer Ltd., Staffordshire, UK) afford the dry hexane, dichloromethane, acetone and methanol extracts.

Anticancer Potency and Cytotoxicity Evaluations of the Extracts against Cancer Cell Lines

The anticancer potency and toxicity potential of the extracts were determined using the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. Yellow water-soluble tetrazolium (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is reduced by metabolically active mitochondrial dehydrogenase enzymes to purple, water insoluble formazan crystals within the cell [20]. The water insoluble formazan crystals dissolves to provide a color change, which can be spectrophotometrically measured and provides an estimate of the number of metabolically active (viable) cells. The relative percentage viable cells were calculated and expressed as percentage cell viability relative to the untreated control (assumed to be 100% viable). All MTT experiments were performed in six-fold (two biological sets of three technical repeats). Whereas the Vero (ATCC® CCL™) green monkey kidney cells, BJ-5ta (ATCC® CRL-4001) Fibroblast immortalized with Human

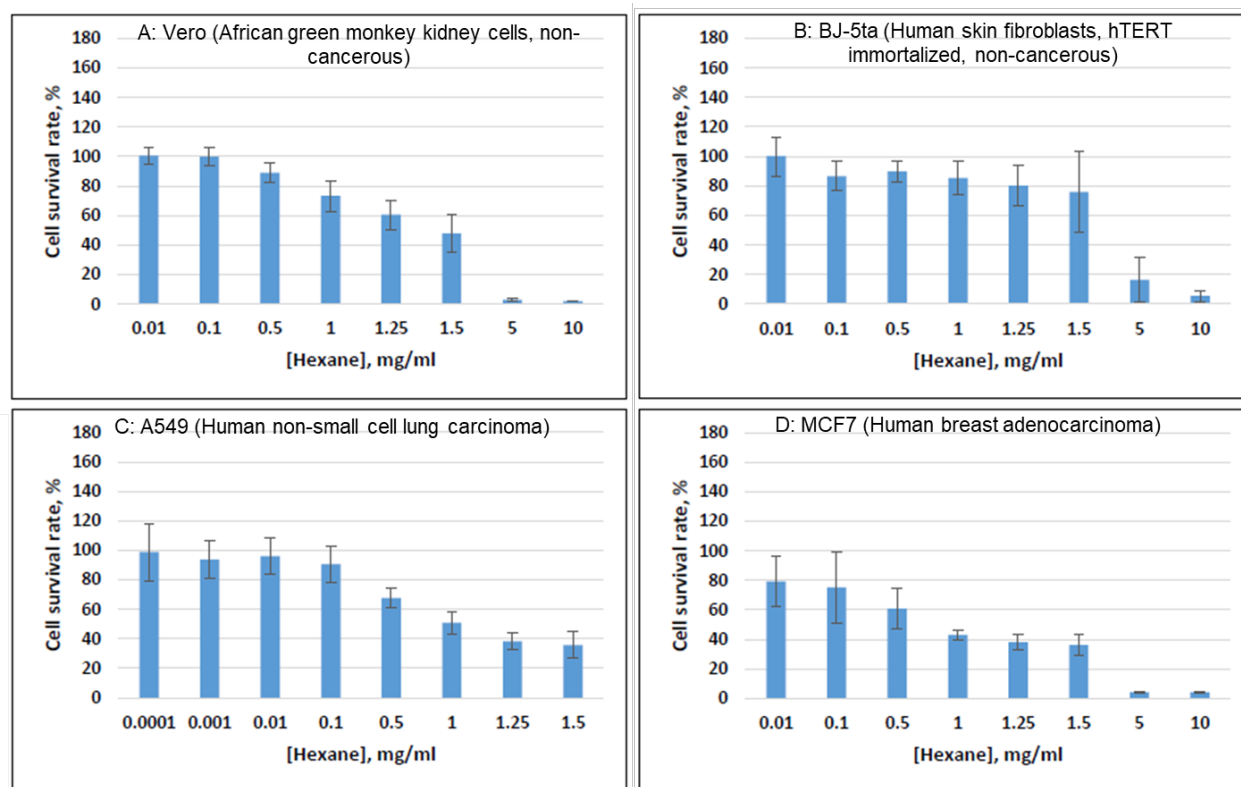


Fig. 1. Cell survival following treatment of Vero cells (A), BJ-5ta cells (B), A549 cells (C) and MCF7 (D) cells for 48 h with *n*-hexane extract, as determined with the MTT assay. MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide).

Telomerase Reverse Transcriptase (hTERT) and MCF-7 (ATCC® HTB22™) human breast cancer were purchased from American Tissue Culture Collection (ATCC, LGC Limited, Middlesex, UK); A549 (#86012804) cells were bought from the European Collection of Authenticated Cell Cultures (ECACC, Sigma-Aldrich, Public Health England, Staffordshire, UK). The purity and identity of each cell lines including A549 (#86012804) was performed by the manufacturer using the American Tissue Culture Collection short tandem repeat (ATCCSTR) where the cell lines were directly purchased and used within early passages. All stocks are meticulously labeled and controlled, and morphology, growth, etc. monitored to ensure integrity of the cell lines. Mycoplasma infection monitoring is performed via fluorescent DAPI nuclei staining a week after initiating culture of each cell stock vial, and then at least monthly thereafter to monitor.

Briefly, cells at different passage numbers were seeded at different densities as indicated in Table 1 and was allowed to incubate for 24 h in 5% carbon (iv) oxide, CO₂ at a temperature of 37 °C, to stick to the surface area.

After which they were treated with each of the test extracts—*n*-hexane, dichloromethane (DCM), acetone, and methanol. The cells were first dissolved in 100% dimethyl sulfoxide (DMSO) (Rochelle & Lab Equipment Cc, Johannesburg, South Africa) and then diluted in complete culture media to 40 mg/mL and 15 mg/mL stock solutions

each, from which working solutions were prepared in complete culture medium to reach a final concentration below 0.625% and 0.2% DMSO (v/v) prior to treatments. At the end of the incubation period, treated cells were rinsed twice with 100 µL Phosphate-buffered saline (PBS), and 200 µL MTT solution at a final concentration of 0.5 mg/mL was added to each well according to the SOP (Pharmac_CTC_SOP023_v02_MTT_Assay). Treated cultures were then incubated for 4 h, after which spent MTT solution was removed and discarded appropriately. 200 µL of DMSO was added to dissolve the water insoluble formazan and the plate was incubated for an additional one hour. Prior concentrations (ranging between 0.0001–10 mg/mL; depending on the cell line) for a duration of 48 h before the cytotoxicity assay was initiated. All the test extracts were first dissolved in 100% DMSO and then diluted in complete culture media to 40 mg/mL and 15 mg/mL stock solutions each, from the stock, was diluted to working solutions were prepared in complete culture medium to afford a final concentration of less than 0.625% and 0.2% DMSO (v/v) prior to treatments.

The plate and its content was placed on a shaker for 15 minutes and absorbance was subsequently measured at, 560 nm cell signal and 630 nm background signals.

As for the experimental groups for MTT, each compound was tested on 3 technical repeats and two biological replicates. 0.2% Triton X-100 (TX-100), known to in-

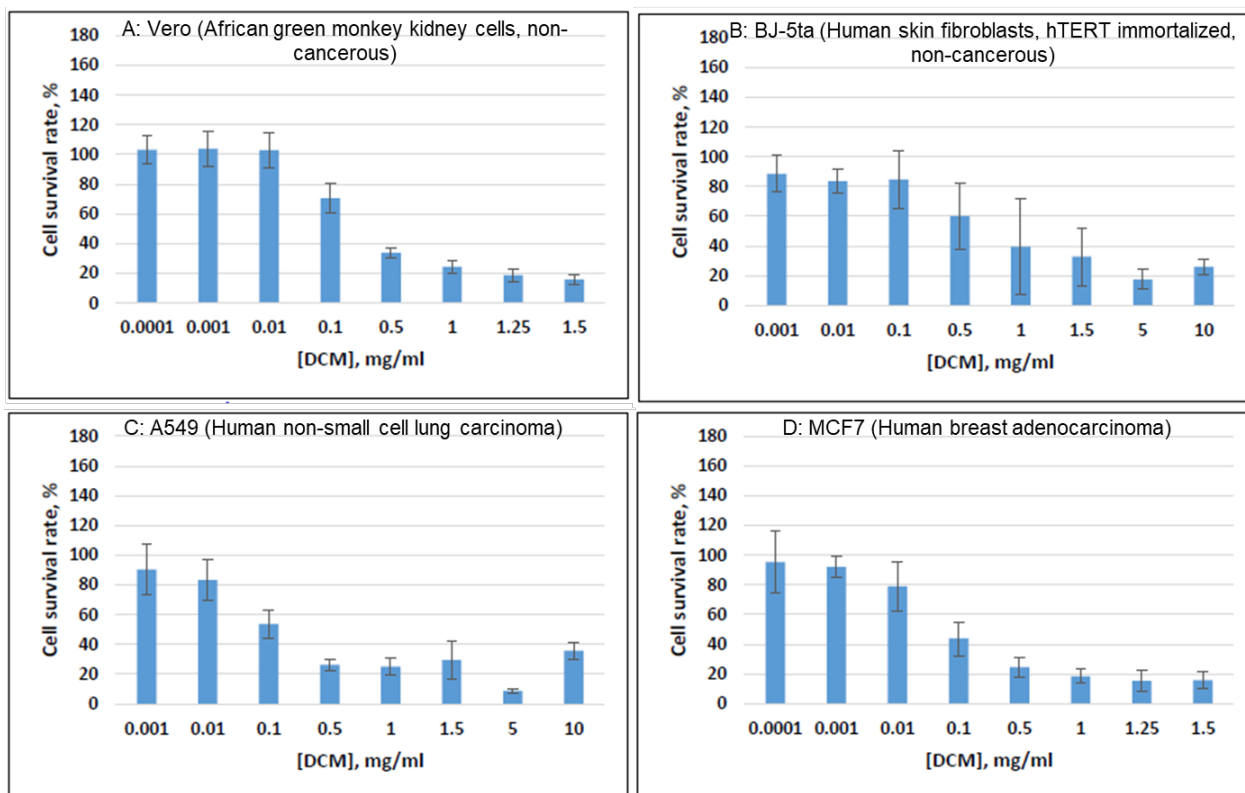


Fig. 2. Cell survival following treatment of Vero cells (A), BJ-5ta cells (B), A549 cells (C) and MCF7 cells (D) for 48 h with dichloromethane (DCM) extract, as determined with the MTT assay.

duce cell-apoptosis [21] was used as an experimental positive control (dead cell control). To measure the effect of TX-100, at the end of the experiment, untreated cells were rinsed following the same method as other experimental groups and exposed to TX-100 for 15 minutes prior to an addition of MTT solution. Cell survival rate expressed relative to the untreated cell control, was determined using the equation below (Eqn. 1).

$$\% \text{ Cell survival rate} = \frac{\Delta \text{ Test sample} - \Delta \text{ Blank}}{\Delta \text{ Control} - \Delta \text{ Blank}} \times 100\% \quad (1)$$

Where, $\Delta \text{Test sample}$ = Test sample absorbance at 560 nm – Test sample absorbance at 630 nm, ΔBlank = DMSO blank at 560 nm – DMSO blank 630 nm and, $\Delta \text{Control}$ = Untreated cells at 560 nm – Untreated cells at 630 nm.

The selectivity index (SI), which indicates the cytotoxic selectivity (i.e., safety of the product) of natural products measured against cancer cell lines versus non-cancerous cells (Vero and BJ-5ta), was calculated using Eqn. 2.

$$\text{Selectivity Index (SI)} = \frac{\text{IC}_{50} \text{ of non-cancerous cells}}{\text{IC}_{50} \text{ of cancerous cells}} \quad (2)$$

GC-MS Analysis

The gas chromatography-mass spectrometry (GC-MS) analysis was conducted using a Shimadzu QP2010 SE (Shimadzu Corporation, Kyoto, Japan) gas chromatograph-mass spectrometer under the following conditions. Inert cap 5MS/SIL, silica capillary column (30 mm $\times$ 0.25 mm ID $\times$ μ mdf, composed of 100% dimethyl-poly-siloxane). Then detection was achieved through the ionization system with ionizing energy of 70 eV. The carrier gas used was helium (99.99%) and kept at a constant flow rate of 1 mL/min; the volume injected was 2 μ L; injector temperature was 290 $^{\circ}$ C and ion-source temperature of 230 $^{\circ}$ C. Isothermally, the oven temperature was set to 50 $^{\circ}$ C for one minute, with a per minute increase of 20 $^{\circ}$ C to 180 $^{\circ}$ C for five minutes, the rate of increase was implemented for five minutes until 240 $^{\circ}$ C and lastly 280 $^{\circ}$ C. The mass spectra were taken at 70 eV, with scan interval of 0.3 sec and fragments from 50 to 700 m/z. GC-MS solutions software version 2.6 was used to handle mass spectra and chromatograms.

Identification of Bioactive Compounds in the Four Extracts

The identification of the anticancer phytochemicals present in the mistletoe extracts were performed by comparing the extracts profiles with those available in the Wiley

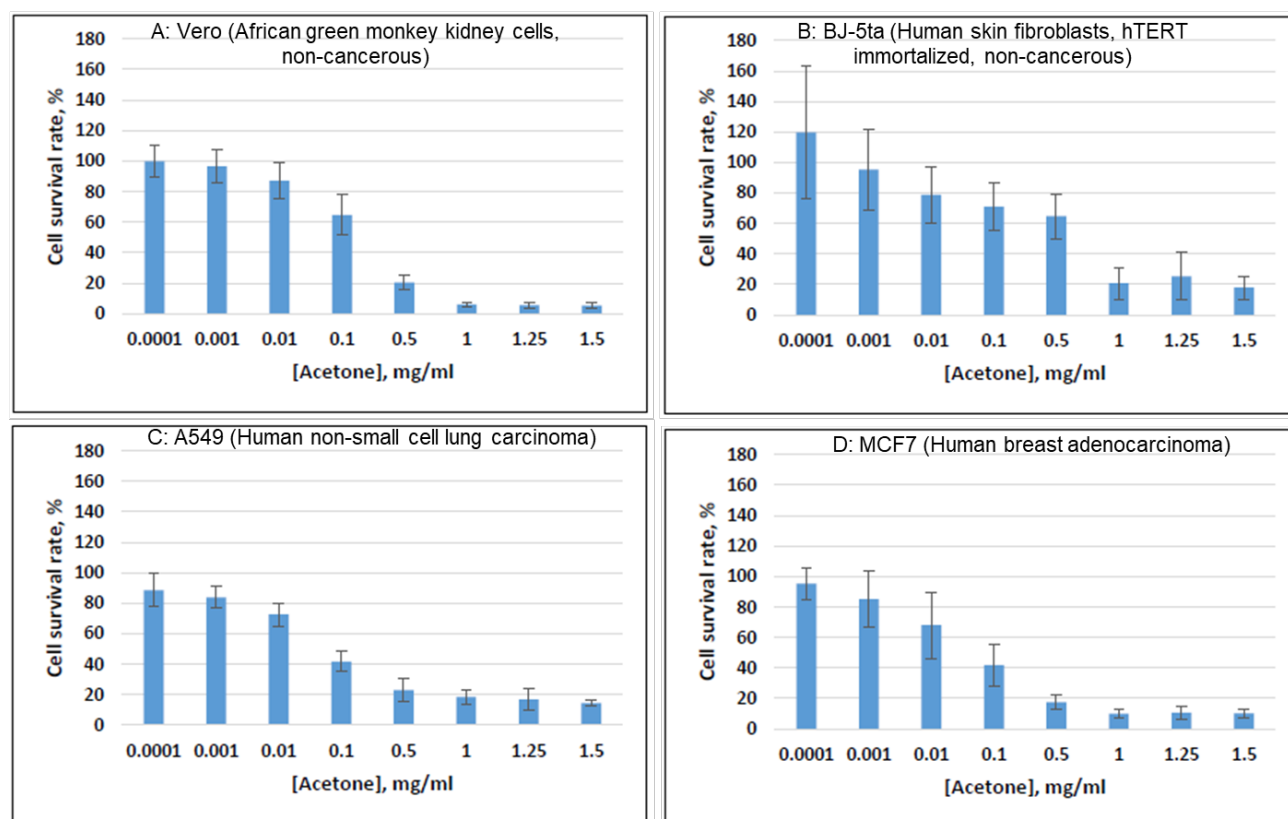


Fig. 3. Vero cells (A), BJ-5ta cells (B), A549 cells (C) and MCF7 (D) relative survival rate after exposure to mistletoe acetone extract for 48 h.

(<https://sciencesolutions.wiley.com/solutions/technique/gcms/>) and NIST (<https://www.nist.gov/>) libraries. An 80% and above match between extracts and library profiles was considered good and used to identify the compounds in the extracts.

Statistical Analysis

SPSS statistical analysis software (Version 25, IBM Analytics, Chicago, IL, USA), in conjunction with the Probit Analysis Method, were used to calculate Half maximal Inhibitory Concentration (IC_{50}) (50% relative viability inhibition) values and 95% confidence limit ranges from the MTT analyses.

Results

Mass and Percentage Yield of the Four Extracts

It is very important to know the number of crude extracts that has been obtained from the process of extraction. As such the mass (% yield) of the extracts after complete dryness were recorded as *n*-hexane—19.23 g (1.92%), dichloromethane—19.04 g (1.90%), acetone—13.13 g (1.32%), and methanol—23.67 g (2.38%).

Cytotoxicity Assay of the Four Mistletoe Extracts

All the four extracts of *Viscum continuun* were completely assayed ($n = 6$) on the four cell lines that form part of this report. The untreated control cells were assumed to have 100% cell viability. Viability for the test groups were expressed relative to this. A vehicle control sample was included for each treatment, consisting of the highest percentage DMSO applied to the cells during treatment (e.g., reach a final concentration $\leq 0.625\%$ DMSO (v/v)). These samples resulted in no cytotoxicity, and any effects of treatment observed was therefore regarded as true representation of the treatment. According to [21] the cytotoxicity of a compound can be categorized in relation to the % Cell viability where: $>80\%$ = non-cytotoxic; $60\%–80\%$ = weakly cytotoxic; $40\%–60\%$ = moderately cytotoxic and $<40\%$ = strongly cytotoxic.

Cytotoxicity of *n*-hexane Extract against the Vero, BJ-5ta, A549 and MCF7 Cell Lines

There was a concentration-dependent decrease in the cell viability of all four cell lines, after 48 h exposure to *n*-hexane extract (Fig. 1A–D). In the Vero non-cancerous cell line (Fig. 1A), the extract appeared non-cytotoxic (viability $>80\%$) up to a concentration of 0.5 mg/mL, weakly cytotoxic (viability $60\%–80\%$) at 1 and 1.25 mg/mL, moderately cytotoxic (viability $40\%–60\%$) at 1.5 mg/mL but

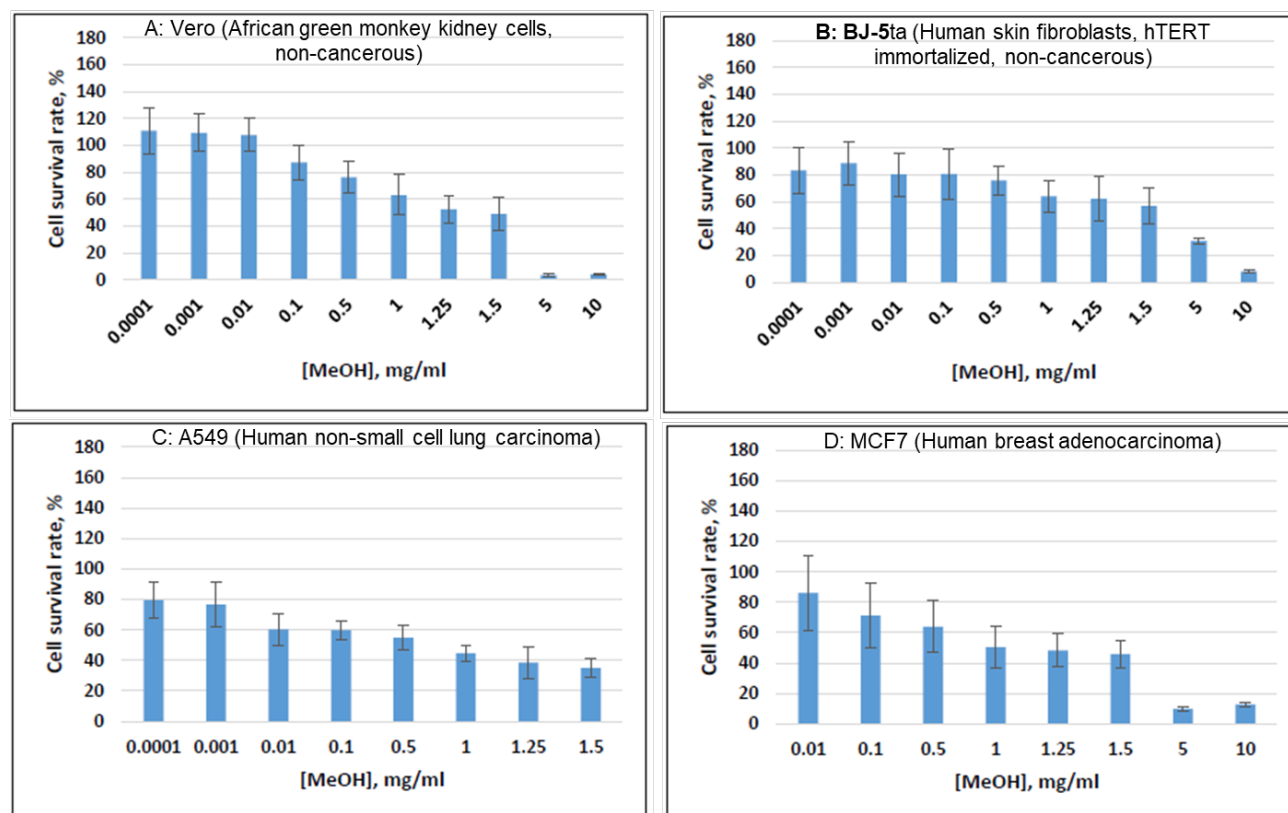


Fig. 4. Vero cells (A), BJ-5ta cells (B), A549 cells (C) and MCF7 (D) relative survival rate after exposure to mistletoe methanol extract for 48 h.

strongly cytotoxic (viability <40%) from 5 mg/mL [14,16]. In the non-cancerous BJ-5ta cells (Fig. 1B), the extract appeared non-cytotoxic (viability >80%) up to a concentration of 1 mg/mL, weakly cytotoxic (viability 60%–80%) at 1.25 mg/mL and 1.5 mg/mL, but strongly cytotoxic (viability <40%) from 5 mg/mL. As for the cancerous A549 cell line (Fig. 1C), the extract appeared non-cytotoxic (viability >80%) up to a concentration of 0.1 mg/mL, weakly cytotoxic (viability 60%–80%) at 0.5 mg/mL, strongly cytotoxic (viability <40%) 1.25 mg/mL. In the cancerous Michigan Cancer Foundation (MCF7) cell line (Fig. 1D), the extract appeared weakly cytotoxic (viability 60%–80%) at 0.01 to 0.5 mg/mL, borderline moderately cytotoxic (viability 40%–60%) at 1 mg/mL, but strongly cytotoxic (viability <40%) from 1.25 mg/mL [20].

Cytotoxicity of DCM Extract against the Vero, BJ-5ta, A549 and MCF7 Cell Lines

Similar to the *n*-hexane exact, the DCM extract also indicated a decrease in the cell viability of all four cell lines that was directly proportional to the extract concentration, after 48 h (Fig. 2A–D). At the highest concentration of 10 mg/mL, DCM extract tended to form a viscous, sticky layer on top of the cells. This viscous layer we thought to have interfered with the absorbance reading and contributed to the slight increase in the percentage cell viability that was

observed. In the Vero non-cancerous cell line (Fig. 2A), the DCM extract appeared non-cytotoxic (viability >80%) up to a concentration of 0.01 mg/mL, weakly cytotoxic (viability 60%–80%) at 0.1 mg/mL, but strongly cytotoxic (viability <40%) from 0.5 mg/mL [22]. In the non-cancerous BJ-5ta cells (Fig. 2B), the extract appeared non-cytotoxic (viability >80%) up to a concentration of 0.1 mg/mL, borderline weakly cytotoxic (viability 60%–80%) at 0.5 mg/mL, but strongly cytotoxic (viability <40%) from 1 mg/mL. In the cancerous A549 cell line (Fig. 2C), the extract appeared non-cytotoxic (viability >80%) up to a concentration of 0.01 mg/mL, moderately cytotoxic (viability 40%–60%) at 0.1 mg/mL, but strongly cytotoxic (viability <40%) from 10 mg/mL. In the cancerous MCF7 cell line (Fig. 2D), the extract appeared non-cytotoxic (viability >80%) up to a concentration of 0.001 mg/mL, weakly cytotoxic (viability 60%–80%) at 0.01 mg/mL, borderline moderately cytotoxic (viability 40%–60%) at 0.1 mg/mL, but strongly cytotoxic (viability <40%) from 0.5 mg/mL [23].

Cytotoxicity of Acetone Extract against the Vero, BJ-5ta, A549 and MCF7 Cell Lines

Continuing the trend, the direct proportionality between extract concentration and the decrease in the relative cell viability of all four cell lines, after 48 h exposure was also exhibited by the acetone extract (Fig. 3A–

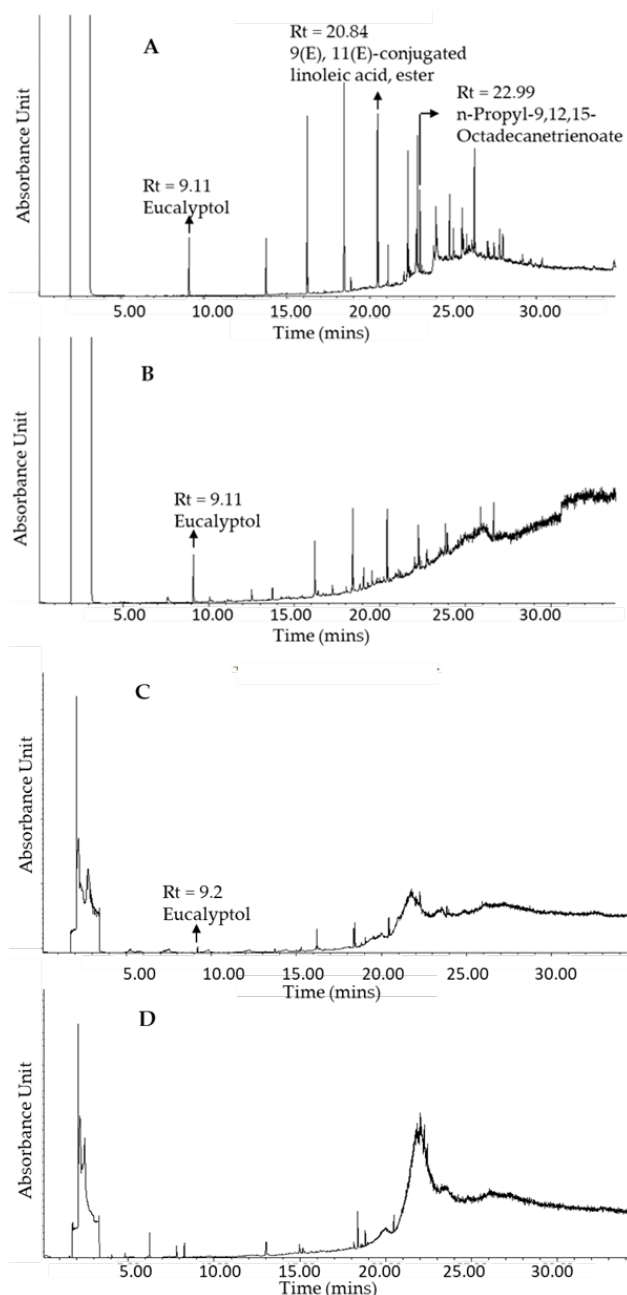


Fig. 5. GC-MS chromatogram of *n*-hexane (A), dichlorormethane (B), acetone (C) and methanol (D) extracts of the mistletoe. GC-MS, gas chromatography-mass spectrometry.

D). In the Vero non-cancerous cell line, the extract appeared non-cytotoxic (viability >80%) up to a concentration of 0.01 mg/mL, weakly cytotoxic (viability 60%–80%) at 0.1 mg/mL (Fig. 3A), but strongly cytotoxic (viability <40%) from 0.5 mg/mL [22]. The lowest concentration seemed to induce slight cell growth in the non-cancerous BJ-5ta cells, with viability increased to more than that of the untreated control (Fig. 3B). In the cancerous A549 (Fig. 3C) and MCF7 cell lines (Fig. 3D), the extract appeared non-cytotoxic (viability >80%) up to a

concentration of 0.001 mg/mL, weakly cytotoxic (viability 60%–80%) at 0.01 mg/mL, moderately cytotoxic (viability 40%–60%) at 0.1 mg/mL, but strongly cytotoxic (viability <40%) from 0.5 mg/mL [22].

Cytotoxicity of Methanol Extract against the Vero, BJ-5ta, A549 and MCF7 Cell Lines

Just as was observed with the other three extracts, relative cell viability of all four cell lines, after 48 h exposure to methanol extract (Fig. 4A–D) was equally a function of the extract concentration. In the Vero non-cancerous cell line, the extract appeared non-cytotoxic (viability >80%) up to a concentration of 0.1 mg/mL (even inducing growth at the lower concentrations), weakly cytotoxic (viability 60%–80%) at 1.0 mg/mL, moderately cytotoxic (viability 40%–60%) at 1.5 mg/mL, but strongly cytotoxic (viability <40%) from 5 mg/mL [11,16] (Fig. 4A). In the non-cancerous BJ-5ta cells, the extract appeared non-cytotoxic (viability >80%) up to a concentration of 0.1 mg/mL, weakly cytotoxic (viability 60%–80%) at 0.5 mg/mL to 1.25 mg/mL, moderately cytotoxic (viability 40%–60%) at 1.5 mg/mL, but strongly cytotoxic (viability <40%) from 5 mg/mL (Fig. 4B). In the cancerous A549 cell line, the extract appeared borderline weakly cytotoxic (viability 60%–80%) at 0.1 mg/mL, moderately cytotoxic (viability 40%–60%) at 0.5 and 1 mg/mL, but strongly cytotoxic (viability <40%) from 1.25 mg/mL [10] (Fig. 4C). In the cancerous MCF7 cell line, the extract appeared non-cytotoxic (viability >80%) at a concentration of 0.01 mg/mL, weakly cytotoxic (viability 60%–80%) at 0.1 and 0.5 mg/mL, moderately cytotoxic (viability 40%–60%) from 1 to 1.5 mg/mL, but strongly cytotoxic (viability <40%) from 10 mg/mL [23] (Fig. 4D).

Half Maximal Inhibitory Concentration (IC_{50}) and Selective Index (SI) of the Four Extracts

The IC_{50} of each extract against the four cell lines was calculated with the Probit statistical analysis method and is summarized in Table 2. The *n*-hexane extract has an IC_{50} of 1.417 ± 0.159 against Vero cells, 2.267 ± 2.59 against BJ-5ta, 1.090 ± 2.592 against A549 and 0.384 ± 0.243 against MCF7. The DCM extract displayed IC_{50} of 0.144 ± 0.104 , 0.435 ± 0.55 , 0.103 ± 0.068 and 0.048 ± 0.038 for Vero, BJ-5ta, A549 and MCF7 cell lines respectively. The acetone extract on the other hand showed some low IC_{50} of 0.055 ± 0.050 against Vero cells, 0.163 ± 0.231 against BJ-5ta, 0.034 ± 0.018 against A549 and 0.020 ± 0.014 against MCF7. And finally the methanol extract displayed IC_{50} of 0.956 ± 0.090 , 1.526 ± 3.96 , 0.251 ± 3.964 and 0.501 ± 0.386 for Vero, BJ-5ta, A549 and MCF7 cell lines respectively. Furthermore, the selective indexes (SI), which enable discernment between toxicity and anticancer activity, were calculated using each of the non-cancerous cell lines and the cancerous cell lines, and is shown in Table 2. For the *n*-hexane extract against A549, it afforded SI of 1.300

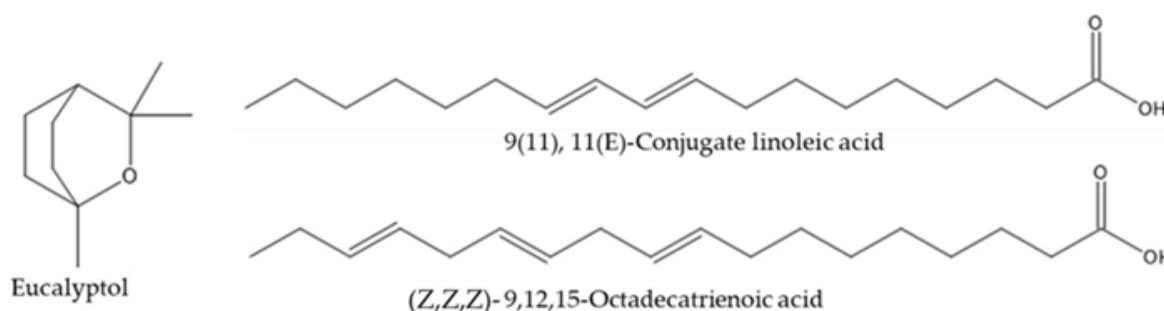


Fig. 6. Structures of three anticancer compounds detected in South African mistletoe extracts. The image was produced by the authors using ChemDraw Ultra 8.0 (Chemistry Software Ltd Gateways, Surrey, UK).

Table 2. The IC₅₀ of each extract against each cell line as calculated using Probit statistical analysis method.

Cell line	<i>Viscum continuum E. Mey. Ex Sprague</i> extracts			
	<i>n</i> -hexane	DCM	Acetone	Methanol
Vero	IC ₅₀ = 1.417 ± 0.159	IC ₅₀ = 0.144 ± 0.104	IC ₅₀ = 0.055 ± 0.050	IC ₅₀ = 0.956 ± 0.090
BJ-5ta	IC ₅₀ = 2.267 ± 2.59	IC ₅₀ = 0.435 ± 0.55	IC ₅₀ = 0.163 ± 0.231	IC ₅₀ = 1.526 ± 3.96
A549	IC ₅₀ = 1.090 ± 2.592	IC ₅₀ = 0.103 ± 0.068	IC ₅₀ = 0.034 ± 0.018	IC ₅₀ = 0.251 ± 3.964
	SI* = 1.300	SI* = 1.393	SI* = 1.618	SI* = 3.809 ^h
	SI [#] = 2.080 ^h	SI [#] = 4.223 ^h	SI [#] = 4.794 ^h	SI [#] = 6.080 ^h
MCF7	IC ₅₀ = 0.384 ± 0.243	IC ₅₀ = 0.048 ± 0.038	IC ₅₀ = 0.020 ± 0.014	IC ₅₀ = 0.501 ± 0.386
	SI* = 3.690 ^h	SI* = 3.000 ^h	SI* = 2.750 ^h	SI* = 1.908
	SI [#] = 5.904 ^h	SI [#] = 9.063 ^h	SI [#] = 8.150 ^h	SI [#] = 3.046 ^h

*Selective index (SI) measured against non-cancerous cell line, Vero cells, [#]Selective index measured against non-cancerous cell line, BJ-5ta cells, ^hHighly selective extracts. IC₅₀, Half maximal Inhibitory Concentration.

and 2.080 when comparing with Vero and BJ-5ta respectively. The *n*-hexane extract has an SI of 3.690 and 5.904 when comparing MCF7 against the Vero and BJ-5ta respectively. The DCM extract against A549 has SI of 1.393 and 4.223 when compared to Vero and BJ-5ta. And on MCF7 the SI are 3.000 and 9.063 respectively against Vero and BJ-5ta. For the acetone extract against A549, it gave SI of 1.618 and 4.794 when comparing with Vero and BJ-5ta respectively. The acetone extract has an SI of 2.750 and 8.150 when comparing MCF7 against the Vero and BJ-5ta respectively. The methanol extract against A549 has SI of 3.809 and 6.080 when compared to Vero and BJ-5ta. And on MCF7 the SI are 1.908 and 3.046 respectively against Vero and BJ-5ta.

Identification of Anticancer Compounds from the four Extracts by GC-MS Analysis

From the GC-MS analysis of the plants, several compound peaks were detected especially for *n*-hexane and dichloromethane (Fig. 5A,B) and less for acetone and methanol extracts (Fig. 5C,D). This was expected because the non-polar *n*-hexane and DCM extract should contain more volatiles compounds. Previous studies proved that eucalyptol has the capability to inhibit the growth of breast cancer cells, MCF7, MDA MB-231 and T47D [24]. Similarly, 9(E),11(E)-conjugated linoleic acid (CLA) refers to a family of 8 geometric isomers of linoleic acid in which

the two double bonds are contiguous. CLA was reported to be effective at reducing mammary tumors in rats at levels as low as 0.1% by weight of their diet [21]. Another compound of interest is (Z,Z,Z)-9, 12, 15-octadecatrienoic acid which has anti-inflammatory, cancer preventive, hepato protective, antioxidant and hypo-cholesterolemic effects [25]. Whereas nearly equal amount of eucalyptol was detected in the non-polar extracts, only trace amount of it was detected in the acetone extract of the plant in this study. 9(E),11(E)-conjugated linoleic acid and is (Z,Z,Z)-9, 12, 15-octadecatrienoic acid were detected only in the *n*-hexane extract (Fig. 5A). We, therefore, propose that amongst other phytochemicals, eucalyptol, 9(E),11(E)-conjugated linoleic acid or its ester and (Z,Z,Z)-9, 12, 15-octadecatrienoic acid detected in South African mistletoe by GC-MS analysis, and their structures shown in Fig. 6, are responsible for the anticancer potentials of the extracts thus validating our earlier mention that the acetone, DCM and the *n*-hexane extracts were highly selective against the breast MCF7 cancer cell line.

Discussion

In the study of medicinal plants extracts or molecules, the importance of establishing cytotoxicity and selectivity index of the extracts or molecules can never be overstated. This is because cytotoxicity studies a pivotal initial step

in determining the potential toxicity of medicinal plant extracts [26]. This is a first time report of the cytotoxicity profile of South African *Viscum continuum E. mey. Ex Sprague* against both cancerous and non-cancerous cells. The results of this study show a concentration dependent cytotoxicity by the four tested extracts across the cell lines tested. The acetone extract appeared to be highly cytotoxic against the non-small cell lung cancer (A549) and breast cancer (MCF7) cell line, purely based on it having the lowest IC₅₀ against the A549 and MCF7 cell lines. The relatively low IC₅₀ value for the acetone extract may be attributed to the saponins, terpenoids, tannins polyphenolic compounds that were detected by the GC-MS analysis. We reported that such compounds (saponins, terpenoids) and (polyphenols, tannins) as moderately and abundantly existing in the acetone extract South African mistletoe extracts [10]. The anticancer potentials of polyphenols and condensed tannins, including gallotannins, ellagitannins, and (-)-epigallocatechin-3-gallate (EGCG) in medicinal plants, and their multiple mechanisms, such as apoptosis, cell cycle arrest, and inhibition of invasion and metastases are well documented. This is in addition to the combinational effects of tannins and anticancer drugs [27]. But since the IC₅₀ concentrations alone may give a skewed view of the cancer selectivity of a compound, the SI ratios were calculated. The selectivity criteria used was based on the previous work conducted, whereby an SI value higher than 2 was considered as highly selective for the cancer type [24,28]. From the SI values, all four extracts showed considerable anticancer effects with highest selectivity against the non-small cell lung cancer, A549 cell line [11]. The acetone and DCM extracts were highly selective against the breast cancer cell line, MCF7, even though the *n*-hexane extract also showed high selectivity towards this cancer line.

Conclusions

The results of this study propose that extracts prepared from the South African Mistletoe tree; *Viscum continuum E. mey. Ex Sprague*, at lower concentrations are not toxic towards cells. They actually show that all four extracts exhibit concentration dependent cytotoxicity and anti-cancer activity as measured against the percentage average cell viability. In addition, three of the extracts were highly selective. However, the methanol extract was selectively more toxic to the lung cancer cell while the other three extracts were highly selectively toxic against breast cancer cell. In conclusion, this study thus validates the use of mistletoe extracts in the management of cancer by rural South Africans. Further studies are needed to ascertain *in vivo* efficacy of the extract in order to elucidate their mechanism(s) of action at molecular and biochemical levels.

Availability of Data and Materials

The data used in the current study are contained within the article.

Author Contributions

Conceptualization, KB; methodology, KB and SM; formal analysis, KB and SM; investigation, SM; writing—original draft preparation, KB and SM; editing, KB; supervision, KB. Both authors have read and agreed to the published version of the manuscript. Both authors have also participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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