

Article

Cytotoxic and Apoptotic Effects of *Leptospermum petersonii*-Derived 6-Methyltectochoyrsin in A375 Melanoma Cells

Johannes Rahlano, Boitumelo Ntisa, Lesetja Motadi and Mpho Choene *

Department of Biochemistry, University of Johannesburg, Johannesburg 2006, South Africa; johannesmatshesa@gmail.com (J.R.); boitumelontisa@gmail.com (B.N.); lmotadi@uj.ac.za (L.M.)

* Correspondence: mchoene@uj.ac.za

Abstract

Background/Objective: Melanoma is the most aggressive form of skin cancer and most common among fair-skinned people, and about 80% of fatalities are from skin lesions. The rise in deaths from melanoma worldwide has been related to a lack of access to healthcare, mostly in low-income nations. Melanoma therapy using current treatments, such as cisplatin, has been beneficial. However, it has been linked to irreversible DNA damage, whereas most current treatments are either ineffective or have intolerable side effects. This study investigated the cytotoxic effects of *Leptospermum petersonii* extracts on A375 melanoma cells and aimed to identify at least one putative bioactive compound. *L. petersonii* is a well-established medicinal plant for its antimicrobial activities, and its leaves are reported to contain essential oils that suppress fungus development. However, no previous research on the effects of this herb on cancer has been reported. **Methods:** The sequential extraction was conducted by soaking the leaves in hexane, ethyl acetate, and methanol. The chemical 6-methyltectochoyrsin was effectively isolated from *L. petersonii* and showed cytotoxicity against melanoma cancer cells. Caspase Glo 3/7 and Annexin V-FITC-PI apoptotic tests were performed following 6-methyltectochoyrsin administration. **Results:** The findings showed cytotoxicity and possible apoptosis induction in A375 cells, and further examination using relative expression analysis revealed an increase in the pro-apoptotic *Bax* and a decrease in the anti-apoptotic *Bcl-2* gene. **Conclusion:** Further research is needed to identify molecular pathways and targets of the compound 6-methyltectochoyrsin in melanoma cancer cells.

Keywords: melanoma; *Leptospermum petersonii*; caspase Glo 3/7; 6-methyltectochoyrsin; apoptosis

Academic Editor: Buyong Ma

Received: 23 June 2026

Revised: 3 August 2026

Accepted: 4 August 2026

Published: 6 August 2026

Copyright: © 2026 by the author. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Cancer remains one of the leading causes of morbidity and mortality worldwide [1], and this prevalence is continuously rising, with most tumors having a poor prognosis [2]. It is a burden of disease worldwide, with around 20.6 million new cancer cases and 9.8 million deaths recorded in 2024, and this is expected to rise to 34.4 million new cases globally by 2050 [3]. Human cancer development is linked to several risk factors, and some of them include exposure to carcinogens like benzene, which has previously been reported to be associated with leukemia development, and Hodgkin's lymphoma (a type of blood cancer). Many physiological activities can generate highly reactive oxygen species (ROS), including H_2O_2 , which can degrade biomolecules like proteins and nucleic

acids [4]. This can lead to the development of degenerative diseases such as cancer, atherosclerosis, and diabetes. Other variables that may contribute to the development of cancer include age, radiation exposure, and genetic alterations [5,6]. Cancer cells develop through an increased proliferation rate and lower apoptosis induction levels, resulting in healthy cells being outnumbered by cancer cells [7].

Skin cancer is a broad term that refers to various malignancies that originate in the skin. Basal cell carcinoma, melanoma, and cutaneous squamous cell carcinoma are examples [8]. Basal and squamous cell malignancies occur on the body's exposed outer layers. They are highly curable if diagnosed and treated early [9]. Melanoma is the most severe and aggressive skin cancer and originates from melanocytes [10,11]. Cutaneous melanomas (CMs) are the most common type of skin cancer among fair-skinned people, and they account for more than 80% of all deaths from skin lesions that are detected late. According to the South African National Cancer Registry, the age-standardized incidence of melanoma among the black African population was 0.5% each year, and the white population incidence was 23.2% of the total population between 2004 and 2015 [12]. In 2024, 337 969 new cases of melanoma were reported worldwide, claiming the lives of 58 398 people [3].

Melanoma is treated with a combination of traditional and targeted therapy. The severe toxicity and lack of efficacy in the later stages of the disease make developing novel medication delivery systems difficult [13]. On the contrary, metastatic melanoma was found to be more resistant to routinely used chemotherapeutic agents. It is frequently challenging to discover novel and efficient cancer treatments [14]. A variety of factors can cause drug resistance, such as excessive expression of the therapeutic target's compensatory mechanism, activation of pro-survival signaling pathways, overexpression of drug transporters, and disruption of apoptotic machinery [15]. Pro-apoptotic drugs, such as cisplatin and 5-fluorouracil, are an effective treatment for melanoma. A platinum derivative that can diffuse into the cytosol is cisplatin. It causes irreversible deoxyribonucleic acid (DNA) damage, which can result in cellular senescence or apoptosis. 5-fluorouracil is a medication that targets the enzymes thymidylate synthase and thymidine monophosphate [16]. However, thymidylate synthase overexpression is highly triggered by treatment in melanoma [17]. The high cost of cancer treatment also poses a challenge that may render treatment inaccessible to people living in developing countries, especially rural areas [18,19]. Hence there is a need to explore alternative cancer treatments that may be more effective, safer, and more affordable [20].

An optimal anticancer agent should be nontoxic, highly effective at lower dosages, and easily accessible at a lower cost. Plants remain a great potential reservoir for bioactive compounds that can be used to overcome the manifestation of many diseases, and most naturally derived compounds are considered to possess less toxic side effects on humans [21]. *Leptospermum petersonii* is an Australian native tree with leaves used for tea, and its presence has also been reported in KwaZulu-Natal in South Africa [22]. *L. petersonii* is a natural source of essential oils used in food, pharmaceutical agents, and cosmetics as it possesses antimicrobial activity. These essential oils also exhibited a high degree of insecticidal activity [23]. *L. petersonii* has also been found to exhibit antifungal activity against *Candida albicans*, *Aspergillus fumigatus*, and most dermatophytes. Therefore, it is suggested to be an alternative therapy for treating fungal infections [24]. The methanolic extract of *L. petersonii* has been reported to exhibit strong antimicrobial activity against *Bacillus cereus* and more potency on *Klebsiella pneumoniae* than well-known ampicillin [25]. Therefore, this study aimed to investigate the cytotoxic effects of *Leptospermum petersonii* extracts on melanoma cancer cells, isolate the possible bioactive compound(s), and further investigate the mechanism of action of the compound(s).

2. Materials and Methods

2.1. Reagents and Equipment

Plant material consisting of the aerial parts of 250 g *L. petersonii* was sampled from February 2007 to January 2008 at a cultivated site in Magoebaskloof, north of Polokwane, Limpopo Province, South Africa. The following solvents were used: hexane and ethyl acetate (Glassworld, Roodepoort, South Africa), methanol (Rochelle Chemicals, Johannesburg, South Africa), dimethyl sulfoxide (DMSO) and etoposide (Merck, Johannesburg, South Africa), silica gel with sieving holes (Merck, Johannesburg, South Africa), A375 melanoma cancer and HEK293 human embryonic kidney normal cells (Japanese Collection of Research Biosources Cell Bank, Ibaraki, Japan), Dulbecco's Modified Eagle Medium (DMEM) and Dulbecco's phosphate-buffered saline (DPBS) (Life Technologies Limited, Paisley, UK), Alamar blue reagent (ThermoFisher, Randburg, South Africa), 1 × trypsin-EDTA (Life Technologies Corporation, Waltham, MA, USA), Synergy HT microplate reader (Biotek, Santa Clara, CA, USA), the Caspase-GLO® 3/7, GoScript™ Reverse Transcriptase System and GoTaq Green Mastermix (Promega Corporation, Madison, WI, USA), CytoSMART™ Lux3FL live imaging cell system (Whitehead Scientific, Cape Town, South Africa), FITC Annexin V/Dead Cell Apoptosis for Flow Cytometry Kit Invitrogen™ (ThermoFisher Scientific, Randburg, South Africa), and ReliaPrep™ RNA Cell Miniprep System (Promega Corporation, Madison, WI, USA).

2.2. Plant Extraction

Dried *Leptospermum petersonii* voucher no:85085-43-4 leaves were soaked in 650 mL Hexane for 24 h, then filtered through a Whatman filter disc. The solvent evaporated, and the leaves were dried then soaked in 650 mL ethyl acetate for 24 h. The extract was filtered into a separate bottle and labeled ethyl acetate extract. The leaves were dried again and immersed in methanol for 24 h. Using a rotary evaporator, the solvents were removed from the extracts, and these extracts were diluted to a final stock solution of 100 mg/mL in 100% DMSO. The hexane extract was not used in any biological activity tests as it did not completely dissolve in DMSO.

2.3. Isolation and Structural Elucidation

The methanolic extract of *L. petersonii* was weighed to 28 g and placed into a 1: 15 (w: v) ratio column chromatography packed with silica gel with sieving holes 60 0.063 0.200 mm. The isolation was carried out in a hexane: ethyl acetate (v:v) gradient polarity percent (5, 10, 20, 30, 50, and 70) of ethyl acetate. The extract was divided into 12 distinct fractions and screened using silica thin-layer chromatography (TLC 60 F254), ultraviolet (UV) detection, and acid stain developing buffer (10% sulphuric acid in dH₂O). The combination with the least complexity was achieved at 20% ethyl acetate. It was then re-columned with 5% ethyl acetate to isolate a needle-shaped yellow component for nuclear magnetic resonance (NMR) and high-resolution mass spectroscopy (HRMS) analysis. The structure of the isolated molecule (6-methyltecto-chrysin) was determined using a combination of HRMS and NMR data processing, as well as a 1-dimensional (¹H and ¹³C) model and a 2-dimensional (¹H and ¹³C) model (COSY—correlation spectroscopy, HSQC—heteronuclear single-quantum coherence, HMBC—heteronuclear multiple-bond coherence). Walters API Quattro Micro spectrometer at the University of Stellenbosch, South Africa, provided the HRMS data. The NMR study was performed in deuterated DMSO with a 500 MHz magnet and an Advance III HD 500 MHz console. For ¹H and ¹³C spectra, the chemical shifts of deuterated DMSO were utilized as a reference, and the internal standard was tetramethylsilane (TMS). To acquire the spectra, the raw data was

processed with Topshim. Comparison with the study in [25] verified the spectrum data obtained.

2.4. Cell Proliferation Assay Using AlamarBlue

A375 melanoma cancer and HEK293 normal cells were extracted at 75% confluency (3rd, 4th, or 5th generation) and resuspended in DMEM at a 5.0×10^4 cells/mL concentration. After that, 100 μ L (5000 cells) was seeded into each well of 96-well plates and incubated for 24 h. The cells in the wells were gently rinsed with DPBS before being treated with various quantities of crude extracts (100 μ g/mL to 0.78 μ g/mL) or 6-methyltectochoyrisin (100 μ g/mL to 1.56 μ g/mL). Cells were treated with 0.1% DMSO as a negative control, DMEM as untreated control, and 100 μ M etoposide as a positive control. The cells were cultured for 22 h, then for 2 more hours with Alamar blue reagent. After 2 h, the fluorescence was measured at emission 530 nm and excitation 590 nm using a Biotek Synergy HT microplate reader. GraphPad Prism version 8.4.3 was used to summarize the results into graphs of biological duplicates and for analysis of variance (ANOVA).

2.5. Caspase-Glo® 3/7 Activity

The cells harvested at 75% confluency (3rd, 4th, or 5th generation) were resuspended in DMEM to a 1.0×10^5 cells/mL concentration, then 200 000 cells (2 mL) were plated into four separate cell culture plates and incubated for 24 h. Following this, the cells were washed gently with DPBS, then treated with either the IC_{50} of 6-methyltectochoyrisin, 100 μ M etoposide, or 0.1% DMSO and incubated for 22 h. Cells with only DMEM served as the untreated sample. The media was collected into separate labeled 2 mL centrifuge tubes as per treatments, and 500 μ L of $1 \times$ trypsin was added into the cell culture plates to collect the adherent cells and then mixed with media. The tubes were centrifuged for 2 min at 532 xg, and the supernatant was discarded. The pelleted cells were rinsed with 500 μ L DPBS before being centrifuged at the same settings, and the supernatant was discarded. The cells were resuspended in fresh media, and 25 μ L of each treatment was collected and transferred into a black 96-well plate, followed by the addition of 25 μ L caspase Glo® 3/7. The plate was covered and shaken for 15 min at room temperature, and the BioTek Synergy-HT plate reader was used to detect relative luminescence units (RLUs). The biological duplicate results were analyzed using GraphPad Prism.

2.6. Brightfield Microscopic Analysis of Cell Morphology

The cells harvested at 75% confluency (3rd, 4th, or 5th generation) were resuspended in DMEM to a 1.0×10^5 cells/mL concentration, then 200 000 cells (2 mL) were plated into four separate cell culture plates and incubated for 24 h. The cells were treated with the IC_{50} of 6-methyltectochoyrisin with all sets of controls included and incubated for 24 h. Following this, images of the cells were captured under brightfield channel (5 milliseconds exposure) and 200 μ m scale bar using CytoSMART™ Lux3FL live imaging cell system.

2.7. Fluorescence Microscopy

Cells extracted at 75% confluency (3rd, 4th, or 5th generation) were resuspended in DMEM to a 1.0×10^5 cells/mL concentration before being plated into four independent wells of a 96-well plate and cultured for 24 h. The cells on the plates were gently rinsed with DPBS before being treated with either the IC_{50} of 6-methyltectochoyrisin, 100 μ M etoposide, 0.1% DMSO, or fresh DMEM as untreated sample for 22 h before Annexin and PI fluorescence. The media was separated into different labeled 2 mL centrifuge tubes according to treatment, and 500 μ L of $1 \times$ trypsin was applied to the cell culture plates to collect adherent cells, which were then combined with the previously collected medium.

The tubes were centrifuged for 2 min at 532 xg, and the supernatant was discarded. The pelleted cells were rinsed with 500 µL DPBS, centrifuged using the same parameters, and the supernatant was discarded. The cells were then resuspended in 100 µL of 1 X Annexin-binding buffer. All fluorescent dyes were applied in accordance with the manufacturer's instructions (FITC Annexin V/Dead Cell Apoptosis for Flow Cytometry Kit Invitrogen™). Fluorescing cells were spotted and counted using the CytoSMART™ Lux3FL live imaging cell system.

2.8. Real-Time Polymerase Chain Reaction

Ribonucleic Acid (RNA) Extraction

RNA was extracted using the ReliaPrep™ RNA Cell Miniprep System. All the solutions were prepared and stored according to the manufacturer's description. The plated cells at a concentration of 1×10^5 cells/mL were either treated with IC₅₀ or IC₉₉ of 6-methyltectoerysin and then incubated for 24 h prior to the RNA extraction process. An untreated control was included. The cells were harvested by trypsinization, centrifuged at 300 xg for 5 min in 2 mL microcentrifuge tubes, and then the supernatant was discarded. The pelleted cells were washed with 500 µL ice-cold DPBS, centrifuged at 300 xg for 5 min, and the supernatant was discarded. The lysates were prepared with 100 µL BL + TG buffer and mixed by vortexing. Following this 35 µL of 100% isopropanol was added and vortexed for 5 s. These volumes were selected from the minimal cell input range of 1×10^5 to 5×10^5 as per the provided protocol. The lysates were transferred to the respective minicolumn tubes and centrifuged at 13 000 xg for 30 s, and flow through was discarded. The minicolumns were washed by adding 500 µL RNA wash solution, centrifuged at 13 000 xg for 30 s, and flow through was discarded. Following this, 30 µL of DNase incubation mix was added directly to the membrane in the minicolumns and incubated for 15 min at room temperature. This was followed by the addition of 200 µL column wash solution and centrifuged at 13 000 xg for 15 s, and then 500 µL RNA wash solution was added and centrifuged for 30 s at the same speed. The minicolumns were then placed on the new collection tubes, and 300 µL RNA wash solution was added into the minicolumns and centrifuged for 2 min at 14 000 xg. The minicolumns were placed on the elution tubes, and 15 µL of nuclease-free water was added directly to the membrane in the column and centrifuged at 13 000 xg for 1 min. The RNA was analyzed using Nano-Drop for qualitative and quantitative checks (A_{260}/A_{280} and A_{230}/A_{260}), and then the RNA samples were aliquoted into a total volume of 2 µL per aliquot in 0.2 mL microcentrifuge tubes and stored at -80 °C.

2.9. cDNA Synthesis Using GoScript™ Reverse Transcriptase

A total concentration of 500 ng/µL RNA solutions were prepared by diluting the RNA samples in a 5 µL RNA primer mix reaction according to Tables 1 and 2 for cDNA synthesis.

Table 1. The RNA-primer mix and respective volumes used for cDNA synthesis.

Components	Volume (Untreated)	Volume IC ₅₀	Volume IC ₉₉
RNA template	1.74 µL	1.43 µL	2.17 µL
Primers [(Oligo (dT15))]	1 µL	1 µL	1 µL
Random primers	1 µL	1 µL	1 µL
Nuclease-free water	1.26 µL	1.57 µL	0.83 µL
Total	5 µL	5 µL	5 µL

The tubes were heated at 70 °C on a heating block for 5 min and chilled on ice while preparing the reverse transcription reaction mix according to the following table:

Table 2. The reverse transcription reaction mix and the respective volumes used for cDNA synthesis.

Components	Volume
GoScript™ 5X reaction buffer	4 µL
MgCl ₂	3 µL
PCR nucleotide mix	1 µL
Recombinant rNasin® ribonuclease inhibitor	1 µL
GoScript™ reverse transcriptase	1 µL
Nuclease-free water	5 µL
Total	15 µL

The reverse transcription reaction mix was combined with the 5 µL of RNA primer mix, and the tubes were transferred into a thermal cycler (BIO-RAD, Hercules, CA, USA). Then the cDNA was synthesized using the following conditions: annealing for 5 min at 25 °C, extension for 1 h at 42 °C, and finally heat-inactivating the transcriptase at 70 °C for 15 min and allowing the thermal cycler to hold the products for 20 min at 4 °C before storing them at −80 °C.

2.10. Real-Time Polymerase Chain Reaction

Table 3 contains primers that were used to amplify each gene for expression analysis using qPCR.

Table 3. Primers used for qPCR.

Gene	Primer Type	Primer Sequence (5'→3')	Molecular Weight (Mw)
GAPDH	Primer 1 (5 nmoles)	5'-ACATCGCTCAGACACCATG-3'	5741.8 Da
	Primer 2 (5 nmoles)	5-TGTAGTTGAGGTCAATGAAGGG-3'	6894.5 Da
β-actin	Primer 1 (5 nmoles)	5'-GTCTTCCCCTCCATCGTG-3'	5377.5 Da
	Primer 2 (5 nmoles)	5'-GATGCCGTGCTCGATGG-3'	5424.2 Da
RBBP6	Primer 1 (5 nmoles)	5'-CAGCTTACAAAGCTGCCAATC-3'	6672.8 Da
	Primer 2 (5 nmoles)	5'-GTAAGATGGAGGTGGTGGAC-3'	6302.1 Da
BAX	Primer 1 (5 nmoles)	5'-AGAGGTCTTTTCCGAGTGG-3'	6179.0 Da
	Primer 2 (5 nmoles)	5'-GCAGTGAGCCCAGATCA-3'	5204.4 Da
BCL2	Primer 1 (5 nmoles)	5'-GCTATAACTGGAGAGTGCTGAA-3'	6823.5 Da
	Primer 2 (5 nmoles)	5'-TGTTAATATCAGTCTACTTCTCTGTG-3'	8206.4 Da
TP53	Primer 1 (5 nmoles)	5'-GACACGCTTCCCTGGATTG-3'	5779.8 Da
	Primer 2 (5 nmoles)	5'-GACGCTAGGATCTGACTGC-3'	5828.8 Da

The amplification of cDNA was measured using SYBR Green-fluorescing dye with the reaction prepared according to Table 4.

Table 4. The SYBR Green master mix used for real-time PCR.

Components	Volume
iTaq Universal SYBR Green Mix 2X	5 µL
Primers (forward and reverse)	1 µL
DNA template	2 µL
Nuclease-free water	2 µL
Total	10 µL

All the tubes with components for the real-time reactions were mixed by spinning down for 5 s and then transferred to a CFX-96 Touch Real-Time PCR System (Bio-Rad) for thermal cycling. The amplification was run as follows:

Initial denaturation was set for 5 min at 95 °C and second denaturation at 95 °C for 15 s. Annealing was kept at 55 °C for 30 s, and extension was 72 °C for 30 s. The system was set for 39 repeats to obtain a total of 40 cycles. Final extension was run for 5 min at 72 °C. Melt curve analysis was done at 95 °C, 65–95 °C for 2–5 s.

The data was exported to an Excel sheet and analyzed using CT formula: $2^{-\Delta\Delta CT}$. The results were summarized into scatter plots using GraphPad Prism.

3. Results

3.1. Chemical Structure and NMR Representation of a Compound Isolated from *L. petersonii*

The yield of 6-methyltectochoyrisin was 235.4 mg (0.84%) from methanolic extract of *Leptospermum petersonii* after conducting multiple runs of column chromatography using hexane: ethyl acetate ratios, a yellow needle-shaped compound with the structure obtained from NMR analysis, Figure 1; δH (500 MHz; 6-methyltectochoyrisin): 12.77 (1H, s, OH), 7.87–7.85 (2H, t, Ar), 7.52–7.47 (2H, s, CH₂), 7.24 (1H, s, SH), 6.64–6.3.62 (1H, br-s, OH), 3.90–3.88 (1H, br-s, OH); δC (125 MHz; 6-methyltectochoyrisin): 182.4, 163.6, 163.5, 158.5, 156.0, 131.6, 131.5, 129.1, 129.0, 126.20, 109.2, 106.0, 105.5, 89.4, 55.9, 7.3. 6-methyltectochoyrisin IUPAC = 5,7-dimethoxy-2-phenyl-4H-chromen-4-one.

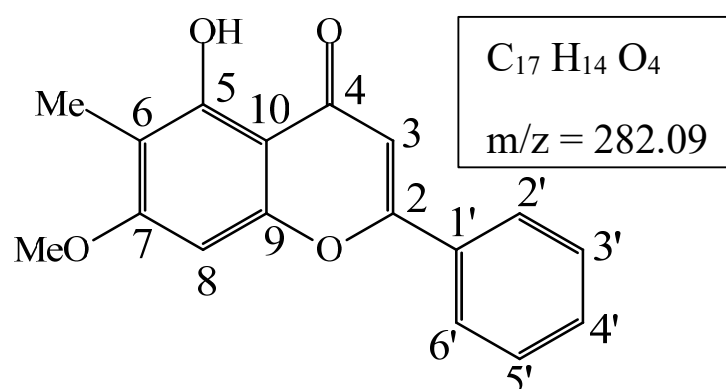


Figure 1. The chemical structure of 6-methyltectochoyrisin. The structure was determined by comparing the proton and carbon spectra (1H and ^{13}C -NMR) to a previously elucidated compound structure. The chemical formula and molecular weight (m/z) were calculated using the ChemDraw version 12.0 software.

There were 15 signals in the spectrum, ranging from δ ppm 7.29 to δ ppm 182.4, with the signals at δ ppm 126.2 and 129.0 integrating for two carbons each (Figure 2). The length of their signal is twice that of $-CH$ signals, suggesting that the symmetry plane exists within the molecule and splits it into two pieces according to the spectrum. This symmetry plane is shown at cyclic B, which allows the NMR to read just a part of the cyclic before creating a signal that is twice as bright as a conventional $-CH$ inside the same molecule. Isoflavonoids have a carbonyl at cyclic C of 182.4 ppm, and C-2 carrying proton has H-2 properties of 163.6 ppm. The carbon assignment (Table 5) was used to extrapolate the ^{13}C NMR data from the spectrum. The data were analyzed to see where the various functional categories stood in relation to one another. The extracted flavonoid was 6-methyltectochoyrisin, as determined by mass spectroscopy.

Table 5. ^{13}C NMR spectral data of re-columned fraction. The spectra of 6-methyltectoerysin were compared against the spectra from [16].

Carbon Position	δ (ppm)	δ (ppm) (Mayer, 1990) [16]
2	163.6	163.4
3	105.9	106.0
4	182.4	182.2
5	158.5	158.3
6	109.2	109.1
7	163.5	163.4
8	89.4	89.4
9	156.0	155.9
10	105.4	105.4
1'	131.5	131.4
2' and 6'	126.2	126.2
3' and 5'	129.0	128.9
4'	131.6	131.6
7-MeO	55.9	55.9
6-Me	7.3	7.4

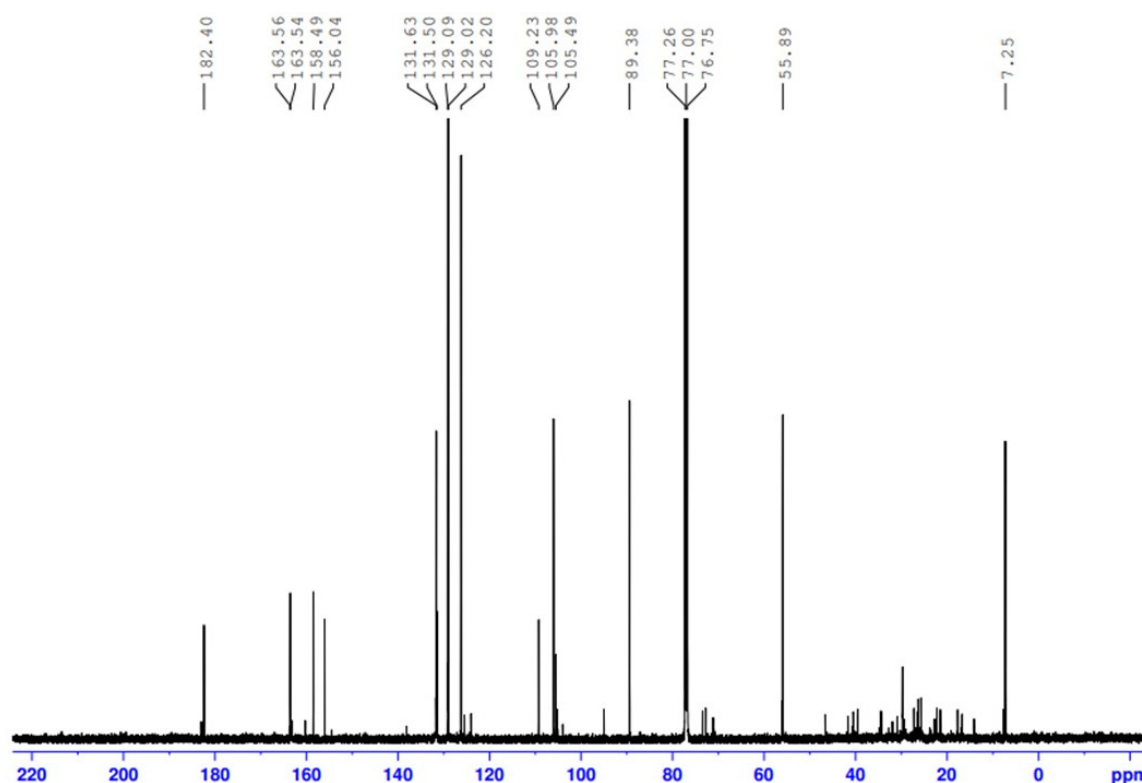


Figure 2. ^{13}C NMR spectrum of 6-methyltectoerysin, showing the chemical shifts between 7.25 and 183 ppm.

3.2. Chemical Identification and Integrity Confirmation Using NMR and HRMS

Each proton and carbon peak was identified using NMR spectroscopy. The number of protons in the 6-methyltectoerysin was confirmed by the ^1H NMR spectrum, and the 2D COSY spectrum indicated the attachment of the phenyl ring at the region of the unsaturated ketone group and coupling of the attached phenyl protons. Due to the long-range coupling between H2' and H6' with the J-value (upper value–lower value in a

doublet) of 8.0 Hz, the 6-methyltectochoyrisin has H3 at 6.63 ppm with doublet multiplicity. The methoxy and methyl peaks were assigned at 3.88 and 2.15 ppm, respectively, after H3 was identified. Because there is no connection with other protons, the H8 proton was discovered at 6.48 using the COSY spectrum. On the HSQC spectra, the OH proton was found at 12.78 ppm and exhibited no connection with any carbon signal. Finally, at 7.90–7.50 ppm, the phenyl protons in the region of the unsaturated ketone group were assigned. The COSY and HSQC spectra (Supplementary File, Figures S1 and S2) proved that the phenyl protons displayed a correlation owing to their respective coupling. Table 5 shows the distinctive peaks indicated by the carbonyl group (C4), C-OH (C5), anomeric carbon (C2), C9, and both methyl protons in the carbon spectrum. Another characterization approach that corroborated these findings is HRMS (Figure 3), which supports the structure of 6-methyltectochoyrisin by validating the molecular mass of 283.0975 g/mol ($M + H^+$) from the HRMS spectrum, which coincides with the theoretical molar mass m/z .

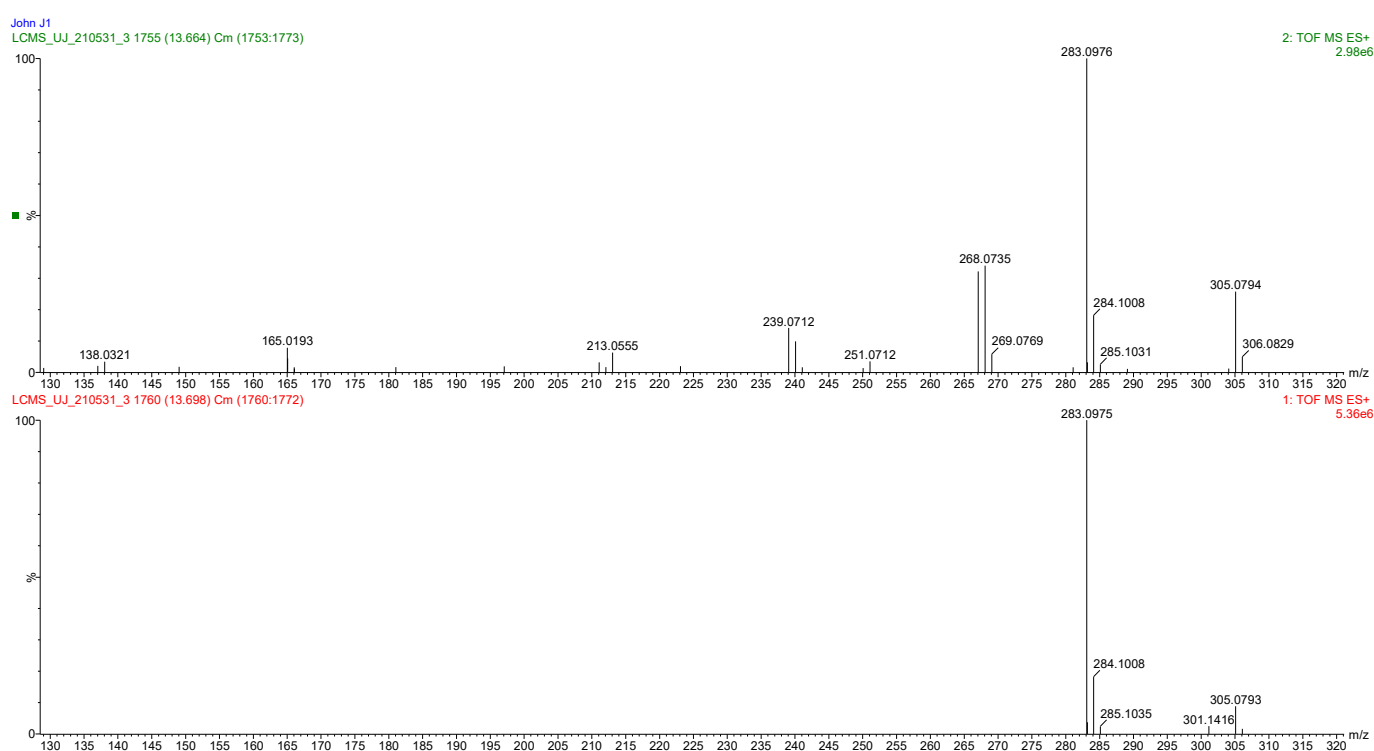


Figure 3. HRMS spectrum of 6-methyltectochoyrisin. The HRMS of the compound shows the signal of the molecular ion at $[M + H]^+$ at 283.0976, which corresponds to $m/z = 282.0976$ and is consistent with the chemical formula of $C_{17}H_{14}O_4$.

3.3. Cytotoxic Activity of the Methanol and Ethyl Acetate Crude Extracts of *L. petersonii* on A375 Melanoma Cancer Cells

The extracts showed significant inhibitory effects of 94.51% (methanol) and 92.09% (ethyl acetate) on A375 cancer cells, and cell viability decreased in a concentration-dependent manner (Figure 4).

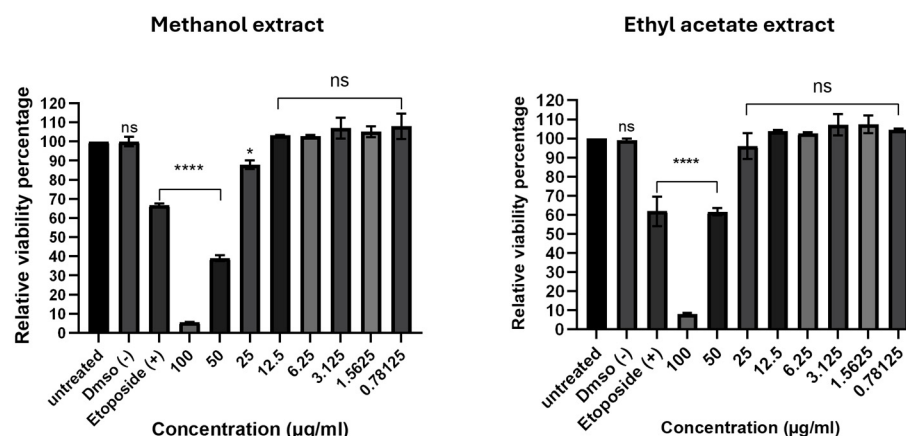


Figure 4. The cytotoxicity of *L. petersonii* extracts (methanol and ethyl acetate) on A375 cancer cells. Data from 2 biological replicates were expressed as mean $\pm$ standard deviation (* $p < 0.05$, **** $p < 0.0001$).

3.4. Cytotoxicity of 6-Methyltectochoyrsin on A375 Melanoma Cancer and HEK293 Normal Cells

6-methyltectochoyrsin was isolated from the methanol extract of *L. petersonii*, and the cytotoxic results of this compound are shown in Figure 5. A375 cells were found to have 24.46% viability and 75.54% inhibition after being treated with the highest concentration of 6-methyltectochoyrsin (100 µg/mL). This inhibitory effect decreased with decreasing concentration, showing concentration-dependent cytotoxic activity of the compound on A375 cells. However, 6-methyltectochoyrsin was toxic to normal cells only at the highest concentration (100 µg/mL), and antiproliferation was not seen from 50 µg/mL of the compound. 6-methyltectochoyrsin had an IC_{50} of 59.32 µg/mL against A375 cells and an IC_{50} of 102.35 µg/mL on HEK293, demonstrating the potency of the compound on cancer cells.

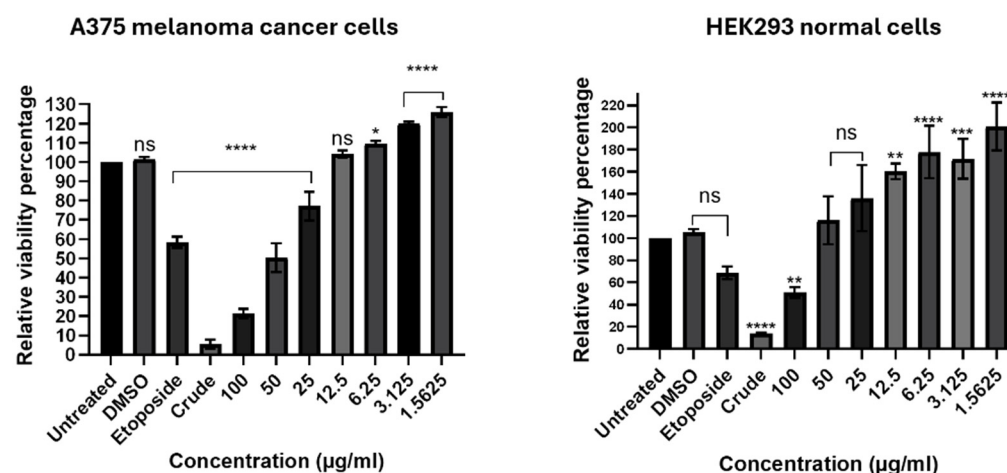


Figure 5. The cytotoxic activity of 6-methyltectochoyrsin on A375 cancer and HEK293 normal cells. Data from 2 biological replicates were expressed as mean $\pm$ standard deviation (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

3.5. Apoptosis Studies Using Caspase 3/7 Activity

Since 6-methyltectochoyrsin possesses antiproliferative activity, caspase 3/7 activity was studied. The luminescence (RLU) of the substrate (caspase GLO 3/7) produced by active caspases was measured relative to the number of apoptotic cells before and after treating the cells with the IC_{50} of 6-methyltectochoyrsin (Figure 6). Treatment of A375 cells with the IC_{50} of 6-methyltectochoyrsin resulted in elevated caspase activity (11,940 RLU),

showing a significant increase in caspase activity in comparison to the untreated cells (2758 RLU).

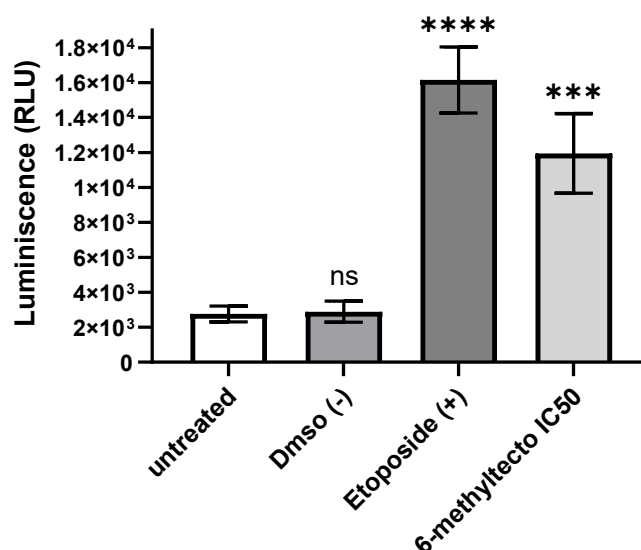


Figure 6. Caspase 3/7 was activated in A375 cells treated with the IC₅₀ (59.32 µg/mL) of 6-methyltecto chrysin. Data from 2 biological replicates were expressed as mean ± standard deviation (***p* < 0.001, *****p* < 0.0001).

3.6. Morphology Analysis of A375 Cancer Cells After Treatment with 6-Methyltecto chrysin

A morphological analysis of A375 cancer cells (Figure 7) was done to confirm the growth inhibition by assessing the growth rate and morphological changes after treatment with 6-methyltecto chrysin. A375 cells assumed their natural epithelial structure in the untreated sample (Figure 7A). However, when treated with the IC₅₀ of the compound, about half of the A375 cells lost their structure, and round-shaped cells were observed (Figure 7D).

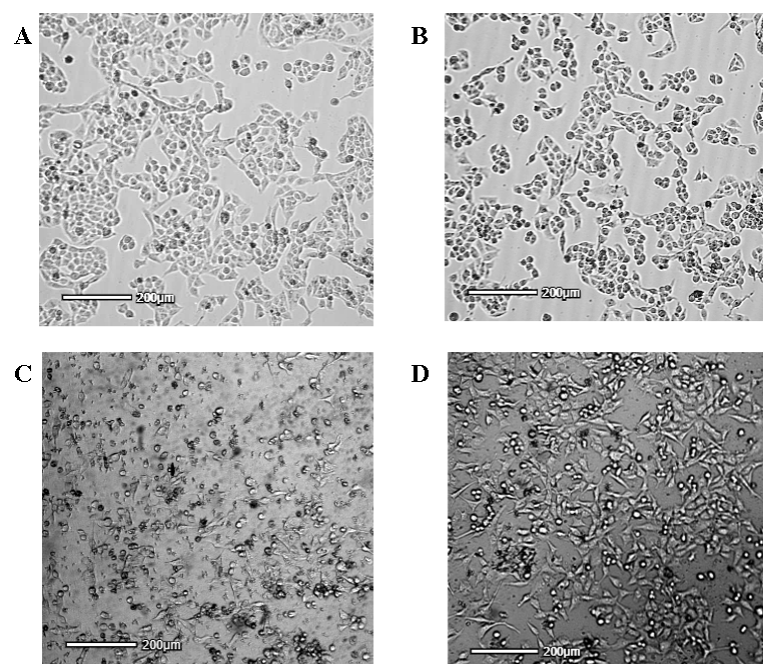


Figure 7. Microscopic images of A375 cells showing morphological changes after being treated with IC₅₀ of 6-methyltecto chrysin and captured with only brightfield (no fluorescence). (A) Untreated cells, (B) 0.1% DMSO, (C) 100 µM etoposide, and (D) IC₅₀ treated cells.

3.7. Apoptosis Analysis Using Fluorescence Microscopy

Apoptosis induction was confirmed using dual labeling with green and red dyes for apoptotic and necrotic cells (Figure 8). This analysis allowed the visualization of fluorescing cells due to the interaction between the Annexin V-FITC and cell membranes. The apoptotic cells were observed with green color, and late apoptotic cells were shown by the high intensity of green color as well as the dual-labeled dyed cells. The number of A375 green-fluorescing (apoptotic) cells observed from untreated (Figure 8A) was low compared to the IC₅₀ treated cells in Figure 8D. The high intensity of green and yellowish dye represents apoptotic cells, and the low intensity of green dye shows live cells undergoing apoptosis.

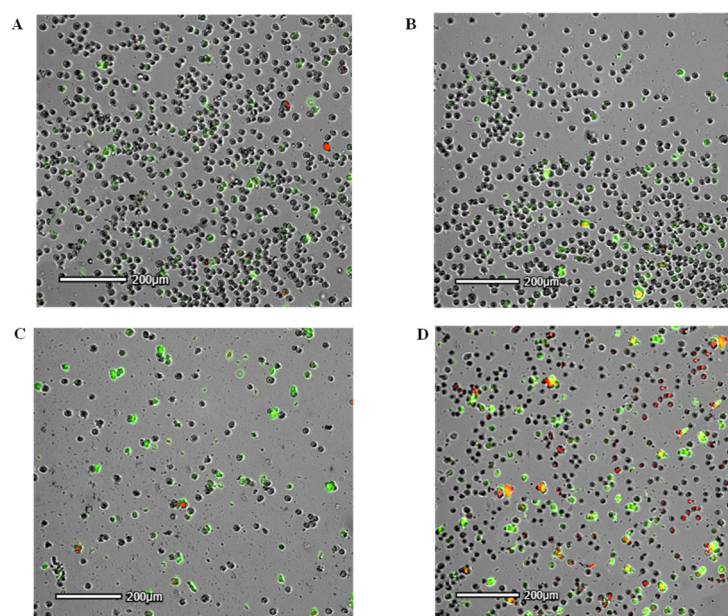


Figure 8. Images of A375 cancer cells after being treated with IC₅₀ of 6-methyltectoerysin. (A) untreated cells, (B) 0.1% DMSO, (C) 100 µM etoposide, and (D) IC₅₀ treated cells. Cells were captured with dual fluorescence and brightfield.

3.8. Real-Time PCR Relative Expression Analysis

The IC₅₀ and the activity of 6-methyltectoerysin on A375 melanoma cancer cells showed potency, thus enabling the progression of this study to assess the apoptotic genes (*RBBP6*, *BAX*, *BCL2*, and *TP53*) using real-time PCR with two housekeeping genes (*GAPDH* and β -actin). The relative gene expression analysis was conducted before and after treating the cells with the IC₅₀ and IC₉₉ of 6-methyltectoerysin (Figure 9). All the genes were analyzed relative to untreated cells, and the positive values of $\log(2^{-\Delta\Delta Ct})$ showed upregulation, whereas the negative ones implicated downregulation. The expression of *RBBP6* and *BAX* was upregulated in both IC₅₀ and IC₉₉ treated cells, whereas *TP53* and *BCL2* were downregulated after treatment.

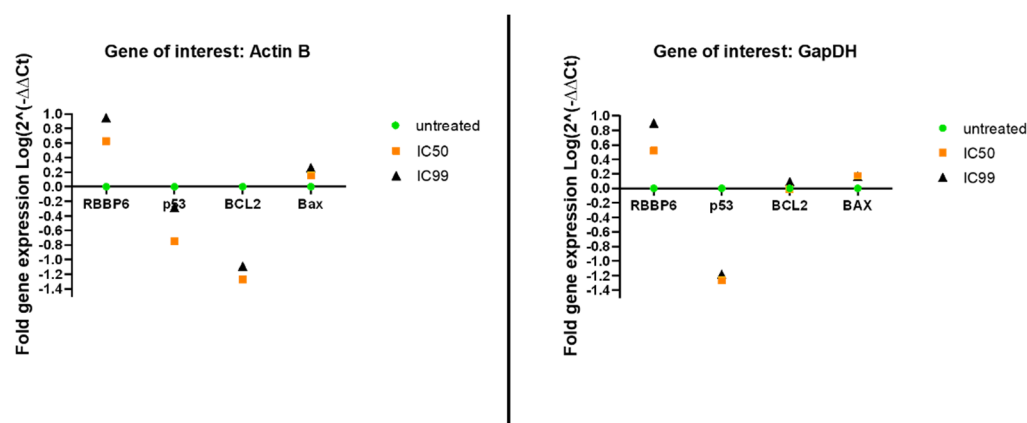


Figure 9. Relative gene expression analysis of apoptosis-related genes before (green circle) and after treating A375 cells with the IC₅₀ (orange square) and the IC₉₉ (black triangle) of 6-methyltectochoyrisin. Fold expression of the genes was analyzed against expression of GAPDH and β-actin housekeeping genes (amplification curves in Supplementary File, Figure S3).

4. Discussion

The study evaluated the effects of *Leptospermum petersonii* on A375 melanoma cancer cells using methanol and ethyl acetate crude extracts. A cytotoxicity study was conducted to determine the most active extract against the cancer cell line, and the methanol extract showed higher inhibitory activity on A375 cells than the ethyl acetate extract. The methanol extract was used for the isolation of the possible bioactive compound, 6-methyltectochoyrisin, which showed potency on A375 cells in a concentration-dependent manner. No anticancer studies have been reported on the methanol extract of *L. petersonii* or on the isolated compound, 6-methyltectochoyrisin. However, the methanolic extract of *L. petersonii* was previously reported to be active against various types of fungi and bacteria [25,26].

The study revealed that the methanolic extract had more potency on A375 cancer cells than the native 6-methyltectochoyrisin, thus suggesting a possible synergistic effect between 6-methyltectochoyrisin and unidentified phytochemicals within the extract. This highlights the importance of molecular networking to understand the interactions between compounds from understudied plants like *L. petersonii*. Ref. [27] revealed that tectochoyrisin, a compound in the same flavonoid class as 6-methyltectochoyrisin, was highly cytotoxic against colon cancer. To compare the activities of tectochoyrisin, 6-methyltectochoyrisin, and other flavonoids, a comparison study on the same cell lines would be conducted.

The study further investigated if the observed cell death resulted from apoptosis as most cancers try to evade this process. Fluorescence microscopy was used to visualize cells undergoing apoptosis, which reacted to specialized dyes, Annexin V-FITC and propidium iodide, two dyes that are commonly used to study different stages of apoptosis [28]. Most cells fluoresced green after treatment with 6-methyltectochoyrisin, indicating the interaction between Annexin V-FITC and phosphatidylserine of the plasma membrane. The exposure of phosphatidylserine from the inner to the outer leaflet of the plasma membrane is a commonly known apoptosis indicator [29,30] since Annexin V-FITC cannot penetrate through the plasma membrane of healthy live cells [31]. Thus, apoptosis was induced after treatment with 6-methyltectochoyrisin. Propidium iodide was used to detect the DNA of dead cells exposed to the surface because the dye cannot diffuse through the plasma membrane of live cells [29]. The green and dual dye-labeled cells represented early and late apoptotic cells, respectively. Cells fluorescing red in the absence of green were cells that died without undergoing apoptosis, and a few were observed in

both untreated and treated A375 cells, possibly due to poor handling during the study. The morphology analysis compared untreated cells with cells treated with 6-methyltecto-chrysin and viewed them under brightfield microscopy to detect the morphological changes. Adherent A375 cancer cells are naturally epithelial [32], which was observed in the untreated cells. However, the cells treated with 6-methyltecto-chrysin assumed changes in their morphology; interestingly, even the quantity of cells observed after treatment was lower than those from untreated conditions.

A caspase activity study further validated that 6-methyltecto-chrysin can activate the effector proteins (caspase 3/7), which play the executioner role in apoptosis induction [33]. Cells undergo cell death in nature; hence caspase activity was detected in the untreated A375 cells. However, the apoptosis rate in cancer may be affected since cancer cells try to evade apoptosis [34]. The IC₅₀ of 6-methyltecto-chrysin significantly increased the caspase activity in the cells in comparison to untreated cells, indicating an increased apoptosis rate. Active caspase 3/7 has a significant function in modulating broad spectra of proteins that aid in eliminating the cells [35]. Therefore, the elevated caspase activity after treatment with the compound demonstrated that apoptosis was the means of cell death.

A successful gene expression analysis was carried out to further investigate the effect of 6-methyltecto-chrysin on other genes involved in apoptotic pathways using real-time PCR. It was employed to study the expression variation of *RBBP6*, *p53*, as well as the *Bax* and *BCL2* ratios in A375 cells before and after treatment with the IC₅₀ and IC₉₉ of 6-methyltecto-chrysin.

RBBP6 appears to regulate the levels of the pro-apoptotic *p53* gene negatively. It is proposed to facilitate cell proliferation by inducing *p53* degradation [36]; therefore, *RBBP6* may be a potential cancer therapy target since it acts as an anti-apoptotic factor [37]. In this study, treatment with 6-methyltecto-chrysin did not suppress the expression of *RBBP6*; instead, the treatments increased the gene expression levels of *RBBP6*. On the contrary, the treatments lowered the *p53* expression levels. Although the *p53* levels did not increase, *p53* showed a biphasic dose–response since IC₅₀ downregulated it more than IC₉₉. There could be other genes involved in apoptosis induction that played a role after treatment with 6-methyltecto-chrysin, and those would require further characterization since the levels of *p53* were low. The pro-apoptotic *Bax* gene was upregulated after treating the cells with 6-methyltecto-chrysin, whereas downregulation of *BCL2* was observed. The pro-apoptotic molecule (*Bax*) is responsible for initiating the release of cytochrome c from the mitochondria [38]. In the case of normal cellular growth (non-cancer cells), the action of *Bax* is blocked by *BCL2* to prevent unwanted cell death [39]. Therefore, the induction of apoptosis after treatment of A375 cells with 6-methyltecto-chrysin may have occurred through the activation of the mitochondrial apoptotic pathway. However, further analysis of protein expression is required to verify the activated apoptotic pathway [40].

5. Conclusions

This study demonstrated that *Leptospermum petersonii* extracts and the isolated flavonoid 6-methyltecto-chrysin exhibit cytotoxic activity against A375 melanoma cells through apoptosis induction. The compound increased caspase 3/7 activity, upregulated *Bax* expression, and downregulated *BCL2* expression, suggesting potential involvement of the mitochondrial apoptotic pathway. Therefore, protein expression analysis is required to verify the activated apoptotic pathway. These findings support the potential of *L. petersonii* as a source of novel anticancer compounds and warrant further mechanistic and in vivo investigations.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/biochem6030021/s1>, Figure S1: Heteronuclear single quantum coherence spectroscopy depicting the single proton–carbon bond correlation within 6-methyltectochoyrisin, with protons represented horizontally and carbons shown vertically; Figure S2: 2D COSY spectrum of 6-methyltectochoyrisin, showing the proton coupling between multiplets; Figure S3: Amplification curves for GAPDH, β -actin, RBBP6, BAX, BCL2, and TP53 at the same concentration of 10 ng/ul cDNA input.

Author Contributions: Conceptualization, L.M. and M.C.; methodology, J.R.; validation, J.R.; formal analysis, J.R. and M.C.; investigation, J.R.; resources, M.C. and L.M.; data curation, J.R. and M.C.; writing—original draft preparation, J.R., B.N. and M.C.; writing—review and editing, B.N., L.M. and M.C.; visualization, J.R. and B.N.; supervision, L.M. and M.C.; project administration, L.M. and M.C.; funding acquisition, L.M. and M.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by The South African Medical Research Council (SAMRC).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data is contained within the article or Supplementary Materials.

Acknowledgments: The authors would like to thank the University of Johannesburg research fund. We would also like to highlight that this work is part of the master’s degree thesis submitted to the University of Johannesburg, Johannesburg, South Africa, in 2022 by J. Rahlano. Title: “Investigating the cytotoxic effects of *Leptospermum petersonii* extracts on melanoma and cervical cancer cells”.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ANOVA	Analysis of variance
BAX	BCL2-associated X
BCL2	B-cell lymphoma 2
cDNA	Complementary DNA
CMs	Cutaneous melanomas
COSY	Correlation spectroscopy
DEPT	Distortionless enhancement by polarization transfer
DMEM	Dulbecco’s Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPBS	Dulbecco’s phosphate-buffered saline
FBS	Fetal bovine serum
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
HEK293	Human embryonic kidney 293
HRMS	High-resolution mass spectroscopy
HSQC	Heteronuclear single-quantum coherence
NMR	Nuclear magnetic resonance
P53	Phosphoprotein 53
PCR	Polymerase chain reaction
PI	Propidium iodide
RBBP6	Retinoblastoma-binding protein 6
RLU	Relative light unit
RNA	Ribonucleic acid
ROS	Reactive oxygen species
TLC	Thin-layer chromatography
TMS	Tetramethylsilane

References

1. Siegel, R.; Naishadham, D.; Jemal, A. Cancer statistics, 2012. *CA Cancer J. Clin.* **2012**, *62*, 10–29.
2. Torre, L.A.; Bray, F.; Siegel, R.L.; Ferlay, J.; Lortet-Tieulent, J.; Jemal, A. Global cancer statistics, 2012. *CA Cancer J. Clin.* **2015**, *65*, 87–108.
3. Sung, H.; Filho, A.M.; Laversanne, M.; Ferlay, J.; Siegel, R.L.; Soerjomataram, I.; Jemal, A.; Bray, F. Global cancer statistics 2024: GLOBOCAN estimates of incidence and mortality worldwide for 34 cancers in 186 countries. *CA Cancer J. Clin.* **2026**, *76*, e70090.
4. Ramalingam, V.; Rajaram, R. A paradoxical role of reactive oxygen species in cancer signaling pathway: Physiology and pathology. *Process Biochem.* **2021**, *100*, 69–81.
5. Niccoli, T.; Partridge, L. Ageing as a risk factor for disease. *Curr. Biol.* **2012**, *22*, R741–R752.
6. Luo, J.; Dai, X.; Hu, H.; Chen, J.; Zhao, L.; Yang, C.; Sun, J.; Zhang, L.; Wang, Q.; Xu, S.; et al. Fluzoparib increases radiation sensitivity of non-small cell lung cancer (NSCLC) cells without BRCA1/2 mutation, a novel PARP1 inhibitor undergoing clinical trials. *J. Cancer Res. Clin. Oncol.* **2020**, *146*, 721–737.
7. Ucar, E.; Özdemir, N.; Altun, E. Fractional order model of immune cells influenced by cancer cells. *Math. Model. Nat. Phenom.* **2019**, *14*, 308.
8. Cullen, J.K.; Simmons, J.L.; Parsons, P.G.; Boyle, G.M. Topical treatments for skin cancer. *Adv. Drug Deliv. Rev.* **2020**, *153*, 54–64.
9. Geraldes, F.O. Pushing the boundaries of computer-aided diagnosis of melanoma. *Lancet Oncol.* **2021**, *22*, 433.
10. Grafanaki, K.; Georgiou, S.; Stratigos, A.J. Solidarity and Voluntarism Amid the COVID-19 Pandemic: Skin Cancer Screening for Blood Donors. *Dermatol. Pract. Concept.* **2021**, *11*, 2021080.
11. Cassano, R.; Cuconato, M.; Calviello, G.; Serini, S.; Trombino, S. Recent advances in nanotechnology for the treatment of melanoma. *Molecules* **2021**, *26*, 785.
12. Gordon, L.G.; Elliott, T.M.; Wright, C.Y.; Deghaye, N.; Visser, W. Modelling the healthcare costs of skin cancer in South Africa. *BMC Health Serv. Res.* **2016**, *16*, 113.
13. Oliveira Pinho, J.; Matias, M.; Gaspar, M.M. Emergent nanotechnological strategies for systemic chemotherapy against melanoma. *Nanomaterials* **2019**, *9*, 1455.
14. Shaffer, S.M.; Dunagin, M.C.; Torborg, S.R.; Torre, E.A.; Emert, B.; Krepler, C.; Beqiri, M.; Sproesser, K.; Brafford, P.A.; Xiao, M.; et al. Rare cell variability and drug- induced reprogramming as a mode of cancer drug resistance. *Nature* **2017**, *546*, 431–435.
15. Su, Z.; Yang, Z.; Xie, L.; DeWitt, J.P.; Chen, Y. Cancer therapy in the necroptosis era. *Cell Death Differ.* **2016**, *23*, 748–756.
16. Wilson, P.M.; Danenberg, P.V.; Johnston, P.G.; Lenz, H.J.; Ladner, R.D. Standing the test of time: Targeting thymidylate biosynthesis in cancer therapy. *Nat. Rev. Clin. Oncol.* **2014**, *11*, 282–298.
17. Serrone, L.; Zeuli, M.; Segal, F.M.; Cognetti, F. Dacarbazine-based chemotherapy for metastatic melanoma: Thirty-year experience overview. *J. Exp. Clin. Cancer Res.* **2000**, *19*, 21–24.
18. Vu, M.; Yu, J.; Awolude, O.A.; Chuang, L. Cervical cancer worldwide. *Curr. Probl. Cancer* **2018**, *42*, 457–465.
19. Shah, S.C.; Kayamba, V.; Peek, R.M., Jr.; Heimbarger, D. Cancer control in low-and middle-income countries: Is it time to consider screening? *Glob. Oncol.* **2019**, *5*, 1–8.
20. Banik, K.; Ranaware, A.M.; Harsha, C.; Nitesh, T.; Girisa, S.; Deshpande, V.; Fan, L.; Nalawade, S.P.; Sethi, G.; Kunnumakkara, A.B. Piceatannol: A natural stilbene for the prevention and treatment of cancer. *Pharmacol. Res.* **2020**, *153*, 104635.
21. Fouché, G.; Cragg, G.M.; Pillay, P.; Kolesnikova, N.; Maharaj, V.J.; Senabe, J. In vitro anticancer screening of South African plants. *J. Ethnopharmacol.* **2008**, *119*, 455–461.
22. Cock, I.E. Medicinal plant images. *Prog. Drug Res.* **2015**, *70*, 179.
23. Park, J.H.; Jeon, Y.J.; Lee, C.H.; Chung, N.; Lee, H.S. Insecticidal toxicities of carvacrol and thymol derived from *Thymus vulgaris* Lin. against *Pochazia shantungensis* Chou & Lu., newly recorded pest. *Sci. Rep.* **2017**, *7*, 40902.
24. Hood, J.R.; Burton, D.; Wilkinson, J.M.; Cavanagh, H.M. Antifungal activity of *Leptospermum petersonii* oil volatiles against *Aspergillus* spp. in vitro and in vivo. *Antimicrob. Chemother.* **2010**, *65*, 285–288.
25. Afolabi, W.O.; Hussein, A.; Shode, F.O.; Le Roes-Hill, M.; Rautenbach, F. *Leptospermum petersonii* as a Potential Natural Food Preservative. *Molecules* **2020**, *25*, 5487.
26. Caputo, L.; Smeriglio, A.; Trombetta, D.; Cornara, L.; Trevena, G.; Valussi, M.; Fratianni, F.; De Feo, V.; Nazzaro, F. Chemical composition and biological activities of the essential oils of *Leptospermum petersonii* and *Eucalyptus gunnii*. *Front. Microbiol.* **2020**, *11*, 409.
27. Park, M.H.; Hong, J.E.; Park, E.S.; Yoon, H.S.; Seo, D.W.; Hyun, B.K.; Han, S.B.; Ham, Y.W.; Hwang, B.Y.; Hong, J.T. Anticancer effect of tectochrysin in colon cancer cell via suppression of NF-kappaB activity and enhancement of death receptor expression. *Mol. Cancer* **2015**, *14*, 124.

28. Kumar, R.; Saneja, A.; Panda, A.K. An Annexin V-FITC—Propidium Iodide-Based Method for Detecting Apoptosis in a Non-Small Cell Lung Cancer Cell Line. In *Lung Cancer*; Humana: New York, USA, 2021; pp. 213–223.
29. Rieger, A.M.; Nelson, K.L.; Konowalchuk, J.D.; Barreda, D.R. Modified annexin V/propidium iodide apoptosis assay for accurate assessment of cell death. *J. Vis. Exp.* **2011**, *50*, e2597.
30. Mahani, M.; Karimi-Mazidi, P.; Khakbaz, F.; Torkzadeh-Mahani, M. Carbon quantum dots—Annexin V probe: Photoinduced electron transfer mechanism, phosphatidylserine detection, and apoptotic cell imaging. *Mikrochim. Acta* **2022**, *189*, 69.
31. Glander, H.J.; Schiller, J.; Süß, R.; Paasch, U.; Grunewald, S.; Arnhold, J. Deterioration of spermatozoal plasma membrane is associated with an increase of sperm lyso- phosphatidylcholines. *Andrologia* **2002**, *34*, 360–366.
32. Ahn, K.H.; Wang, E.J.; Kim, J.; Han, S.H.; Jang, J.H.; Kim, H.Y.; Cho, G.J.; Hong, S.C.; Oh, M.J.; Kim, H.J. Effects of genistein, genistin, and daidzein on preterm Drosophila model and human cervical epithelial cells. *Am. J. Obstet. Gynecol.* **2022**, *226*, S516–S517.
33. Araya, L.E.; Soni, I.V.; Hardy, J.A.; Julien, O. Deorphanizing Caspase-3 and Caspase-9 Substrates In and Out of Apoptosis with Deep Substrate Profiling. *ACS Chem. Biol.* **2021**, *16*, 2280–2296.
34. Thapa, S.; Rather, R.A.; Singh, S.K.; Bhagat, M. *Insights into the Role of Defective Apoptosis in Cancer Pathogenesis and Therapy*; IntechOpen: London, UK, 2021.
35. Christgen, S.; Place, D.E.; Kanneganti, T.D. Toward targeting inflammasomes: Insights into their regulation and activation. *Cell Res.* **2020**, *30*, 315–327.
36. Mbita, Z.; Hull, R.; Mbele, M.; Makhafa, T.; Dlamini, Z. Expression Analysis of RBBP6 in human cancers: A Prospective biomarker. *Anti Cancer Drugs* **2019**, *30*, 767–773.
37. Pretorius, A.; Kaur, M.; Wamalwa, M.; February, M.F.; Essack, M.; Bajic, V.B.; Rees, D.J.G. Functional analysis and characterization of the human RBBP6 promoters based on a combination of molecular biology and in silico approaches provide additional evidence for RBBP6 role in apoptosis. *J. Biosci.* **2013**, *1*, 2251–3159.
38. Ansari, M.Y.; Ball, H.C.; Wase, S.J.; Novak, K.; Haqqi, T.M. Lysosomal dysfunction in osteoarthritis and aged cartilage triggers apoptosis in chondrocytes through BAX mediated release of Cytochrome c. *Osteoarthr. Cartil.* **2021**, *29*, 100–112.
39. Lu, C.; Yang, D.; Sabbatini, M.E.; Colby, A.H.; Grinstaff, M.W.; Oberlies, N.H.; Pearce, C.; Liu, K. Contrasting roles of H3K4me3 and H3K9me3 in regulation of apoptosis and gemcitabine resistance in human pancreatic cancer cells. *BMC Cancer* **2018**, *18*, 149.
40. Rahlano, J. Investigating the Cytotoxic Effects of Leptospermum petersonii Extracts on Melanoma and Cervical Cancer Cells. Master’s Thesis, University of Johannesburg Library, Johannesburg, South Africa, 2022.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.