

Evaluation of Antiproliferative Activities of an Isolated Compound from *Punica granatum* Seed against Non-Small Cell Lung Adenocarcinoma Cell Line (A549)

Samba S. Nelson^{1,2}, Sangilimuthu Alagar Yadav^{1,*} , Ravi Subban³

¹ Department of Biotechnology, Centre For Natural Products and Functional Foods, Karpagam Academy of Higher Education, Coimbatore- 641 021, India

² Department of Medical Laboratory, Kampala International University, Tanzania

³ Department of Chemistry, Karpagam Academy of Higher Education, Coimbatore- 641 021, India

* Correspondence: sangilimuthu.a@kahedu.edu.in

Received: 7.03.2024; Accepted: 22.07.2025; Published: 30.09.2025

Abstract: Tri-O-punicyl glycerol (PG-1) was isolated and characterized from the ethyl acetate extract of *Punica granatum* seed, and it has an antiproliferative effect against lung cancer cell lines (A549), which has no toxic effects on fibroblast cell lines (normal cell line). Also, the ethyl acetate extract of *P. granatum* contains twelve volatile phytoconstituents, including octadecatrienoic acid as a major compound (19.8%) by GC-MS analysis. The IC₅₀ value of PG-1 and standard drug (doxorubicin) on the A549 cell line was found at 25±8.5 µg/ml and 22±7.4 µg/ml, respectively, through MTT assay. At a dose of 250µg/ml of PG-1, the comet test revealed DNA damage, and flow cytometry revealed a cell cycle arrest at the G0 phase at a concentration of 12 µg/ml of PG-1. PG-1 is also an efficient antioxidant compared to L-ascorbic acid, as determined by DPPH radical scavenging activity found in the IC₅₀ value at 26±5.7 µg/ml and 24±6.8 µg/ml, respectively. In the meantime, we have observed the ABTS radical scavenging activity of PG-1 and L-ascorbic acid and found the IC₅₀ values of 26±0.62 µg/ml and 23±0.65 µg/ml, respectively. PG-1 as a potential lead compound from *P. granatum* against the lung cancer cell line (A549) was achieved in this study. Further, the active mechanism of Tri-O-punicyl glycerol for lung cancer treatment needs to be evaluated in future clinical trials.

Keywords: *Punica granatum*; tri-O-punicyl glycerol; anticancer; lung cancer; cell line.

© 2025 by the authors. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. The authors retain copyright of their work, and no permission is required from the authors or the publisher to reuse or distribute this article, as long as proper attribution is given to the original source.

1. Introduction

Lung cancer is considered a global threat due to its high fatality rates. The United States of America has the most cases, and it has become one of the most lethal diseases for both men and women in India [1]. Abraxane is used as first-line therapy for non-small cell lung cancer [2]. Due to the severe adverse effects triggered by synthetic medications, researchers have turned to herbal-based medicine as an alternative medicine and have begun testing isolated compounds against lung cancer [3]. Numerous medicinal plants and their active constituents are reported against various types of lung cancers. Also, medicinal plant combinations play a vital role in treating cancer with fewer side effects. James et al. [4] reported that combining active compounds from Himalayan plants through nanocarrier-mediated drug delivery for

cancer treatment produces natural drugs with less side effects and enables an effective targeted delivery of drugs. It reduces drug resistance in cancer treatment. The medicinal plants reported against lung cancer are *Curcuma longa*, Grapes, *Panax ginseng*, *Peganum harmala*, and *Piper* species. These medicinal plants effectively inhibited lung cancer due to the presence of curcumin, Ginseng, beta-carotene, and piperine, respectively [4].

Punica granatum L. is a well-known plant that attracts the public and researchers because of its health advantages and the beneficial effects of its active principles against a variety of diseases and disorders. Due to the inclusion of active substances, it has many pharmacological properties such as antioxidants, lowering blood sugar and fats, preventing cancer cell proliferation, and anti-mutagenic and anti-carcinogenic effects. *P.granatum* L consists of fumaric acid, vitamins C, Vit B1, Vit B2, beta carotene, gallic acid, malic acid, tartaric acid, succinic acid, oxalic acid, citric acid, chlorogenic acid, ellagic acid, coumaric acid, cinnamic acid, hydroxy benzoic acid, hydroxy protocatechuic acid, caffeic acid, kaempferol, ferulic acid, pellettierine, punicalagin, quercetin, catchin, phloridzin, anthocynins, luteolin, narigenin and punicalin [5]. Still, some parts of *Punica granatum* L., including seeds and its active phytomolecules against various diseases, such as cancer, need to be investigated.

Among the primary biomedical properties of pomegranate, anti-cancer and immunomodulatory actions are believed to be promising study areas [6]. In a cell line investigation, pomegranate fruit extract inhibited cell proliferation and angiogenesis indicators such as MAPK, NF-kappa B, and PI3K/Akt in a human cancer cell line (A549). Pomegranate fruit extracts inhibited tumor cell proliferation and development effectively against breast cancer [7]. In an animal study, the effect of pomegranate extracts on the protective action against methotrexate-induced lung injury, it was found that using pomegranate extracts as a prophylactic substantially decreased the total oxidant status and the oxidative stress index while increasing total antioxidant capacity. This shows that pomegranate extracts could be used as an adjuvant in lung cancer treatment [8,9]. Many studies on pomegranate extracts have been reported by various authors. However, there is still a gap in identifying the active molecules in pomegranate research, particularly extracts of pomegranate against different illnesses through clinical trials, as well as the isolation of active molecules from pomegranate seeds. It must address the requirements of society in useful areas, including pharmacology and drug development. Previous research in our lab found that there are higher antioxidant and anticancer properties in the ethyl acetate extract of pomegranate seed than in the methanol extract [10]. The present research focuses on isolating and characterizing active molecules present in the ethyl acetate extract of pomegranate seed against the lung cancer cell line (A549) through MTT assay, flow cytometry analysis, and Comet assay.

2. Materials and Methods

2.1. Collection and extraction of the plant sample.

The *P.granatum* fruit was acquired in Coimbatore, Tamil Nadu, in May 2021. The fruit (1 kg) was sliced into small pieces, and the seeds were collected after the exocarp and mesocarp parts had been eliminated. After removing the juicy liquid part of the seeds, they were dried for 3-5 days at room temperature. The dried seed (100 g) was ground to powder and extracted exhaustively for 12 hours with ethyl acetate in a 1:5 (w/v) ratio employing a Soxhlet apparatus with 5-6 successions. The extract was dried with a rotary flash evaporator to get a solid product

(10 g) for further fractionation and biological activity research [10]. Sigma Aldrich provided the solvents and chemicals used in this study.

2.2. Cell culture and maintenance.

The lung cancer cell line (A549) was obtained from the National Centre for Cell Science (NCCS) and cultured in Eagle's Minimum Essential Medium with 10% fetal bovine serum (FBS). The cells were kept at 37°C, 5% CO₂, 95% air, and 100% relative humidity, and the culture medium was replaced twice a week. Cultures were passaged once a week for maintenance.

2.3. Gas chromatography-mass spectrum (GC-MS) analysis.

The volatile components of an ethyl acetate extract of *P.granatum* seeds were also determined using GC-MS [11]. 1 l of the extract was diluted in petroleum ether before being injected in the split mode. For the analysis, a split ratio of 10:1 was used. The Perkin Elmer-Clarus 500 was utilized for the analyses, using a capillary column directly attached to the mass spectrometer system column (Elite-5ms) (30mx 0.25mm ID fused of 5% phenyl 95% dimethylpolysiloxane). Oven temperature 70°C @ 6°C minutes to 170°C (2 min) @ 6°C/min to 290°C (5min); injector temperature 290°C, Helium as a carrier gas and 1ml/min flow rate; mass spectral data was collected at 70ev, 200°C, scan.

2.4. Isolation of active phytochemical from *P.granatum* using column chromatography.

Column chromatography was used to separate bioactive components (PG-1) from *P.granatum* seed. The column was packed with silica gel and petroleum ether as the solvent. 10 g of ethyl acetate extract from *P.granatum* seed was put onto the column and eluted with petroleum ether and a combination of petroleum ether and ethyl acetate in increasing polarity. TLC was used to monitor fractions of 50 ml collected, concentrated to remove the solvent, and monitored using petroleum ether: ethyl acetate (8:2) as the eluting solvent. Five fractions were recovered and discarded after eluting with pure petroleum ether. The elution was resumed with an 8:2 petroleum ether: ethyl acetate combination. Overall, 45 fractions were collected, and each fraction was monitored by TLC. The 11th fraction from petroleum ether: ethyl acetate (8:2) combinations produces a single spot in TLC. Further, it is taken for spectral analysis. After solvent removal, the eleventh fraction revealed a yellow-colored (440 mg) PG-1 that was homogeneous by TLC and showed a single spot with an R_f value of 0.76. Further, the UV-Vis spectral data, FT-IR spectral data, ¹³C NMR, and ¹H NMR spectral data of PG-1 were used to characterize its chemical structure and molecular formula.

2.5. Evaluation of in vitro antioxidant activity of isolated compound using DPPH and ABTS assay.

The antioxidant profile of PG-1 derived from *P.granatum* seed was examined using 0.1mM DPPH as radicals. As the standard antioxidant, L-ascorbic acid was used. For this study, PG-1 was dissolved in MeOH at concentrations ranging from 0 to 85 µg/ml and treated on 0.1mM DPPH. After adding the extract, the peak color reduction of DPPH, indicating the higher free radical scavenging capacity of the extract, was observed at 517 nm. DPPH scavenging activity was calculated with the following formula : 1.

$$\% \text{ inhibition} = \frac{(\text{Control} - \text{Sample})}{\text{Control}} \times 100 \dots \dots \dots (1)$$

The ABTS radical scavenging activity of PG-1 was evaluated using a 7.6 mM ABTS solution with a 2.6 mM potassium persulfate solution. After 12 hours of incubation, 3 ml of preprepared ABTS solution was treated with different concentrations (6, 12, 25, 55, and 85 µg/ml) of PG-1, and the color reduction was measured at 734 nm after one minute of incubation at room temperature using a spectrophotometer (Shimadzu-2450, Japan) [12,13]. The percentage inhibition was calculated using the above formula 1.

2.6. Antiproliferative ability of the isolated compound using MTT assay.

MTT assay was used to assess the antiproliferative ability of PG-1 against the lung cancer cell line (A549) [14]. The cells were cultivated in 96-well tissue plates and then treated with PG-1 at doses of 6, 12, 25, 55, and 85 µg/ml, with doxorubicin serving as a positive control. The cells were treated according to the experimental design, and the incubation period was optimized for each cell type and system. MTT (5mg/ml), a tetrazolium chemical, was added to the wells and incubated. MTT was converted to insoluble purple formazan dye crystals by metabolically active cells. The detergent was then applied to the wells to dissolve the crystals, and the absorbance was measured using a spectrophotometer at 570 nm. The data was analyzed by plotting extract concentration vs absorbance, which allowed quantification of alterations in cell growth and % inhibition. The formula 2 below was used to calculate the percentage of cell inhibition.

$$\text{Inhibition of cells} = 100 - \text{Abs (sample)}/\text{Abs (control)} \times 100 \dots \dots \dots (2).$$

A nonlinear regression graph was generated between % inhibition of the cell to determine the 50% inhibitory concentration of samples [15].

2.7. Determination of cell cycle arrest of the PG-1 from P. granatum seed extract on lung cancer cell line (A549) by Flow cytometry.

Cell cycle analysis of PG-1 was performed on the A549 cell line to determine the cell cycle arrest of PG-1 from the ethyl acetate extract of *P.granatum*. The analysis procedure was done primarily by cell seeding in 6-well plates with a cell density of 2×10^5 cells. The cells were then treated with 120 µg/ml (Low dose) and 250 µg/ml concentrations of isolated PG-1 (High dose IC_{50}). The cells were trypsinized and centrifuged at 100 rpm for 10 min after incubation. Then, the cells were washed three times with PBS, fixed in 70% ice-cold ethanol, and stored at 4°C. The cells were stained with 50 µL of propidium iodide after fixation and stored in a dark room at room temperature for 30 minutes. The propidium iodide chemical was visualized using a Semrock fluorescence block filter (595-615 LF561-A; Semrock, Inc., Rochester, NY). Flow cytometry on the BD FACS Canto II device, data analysis with the FlowJo software. Then, the cells were analyzed with a flow cytometer (BD Accuri™ C6, USA), and the flow jow program was used to determine cell cycle dispersion [16,17].

2.8. Genotoxicity studies of PG-1 on lung cancer cell lines (A549).

Genotoxicity of PG-1 on lung cancer cell lines (A549) was assessed with different concentrations of PG-1 (0, 50, 120, and 250 µg/mL) for 48 hours in 96-well plates. Cells were

obtained after incubation, and DNA damage was assessed using the Comet test [18]. Soaking of slides in two lysis solutions for thirty minutes at the ambient temperature. The first solution is 0.4 M Tris-HCl (pH 7.5) and 1% SDS, while the second solution is 0.4 M Tris-HCl (pH 7.5), 1% SDS, and 0.05 M EDTA. The slides were washed in 1%TBE buffer (89 mM Tris, 89 mM boric acid, with 2.5 mM EDTA, pH 8.3), a ten-minute solution of fresh water was used for treatment (0.03 M NaOH, 1 M NaCl) for 2.5 minutes to cleave alkali-labile sites. The slides are placed horizontally in electrophoresis solution on a tray filled with fresh electrophoresis solution (0.03 M NaOH, pH 13). The electrophoresis was performed at 20 V for 12.5 minutes at room temperature. Slides were gently removed from the tray, and a neutralizing solution (0.4 M Tris-HCl, pH 7.5) was used to wash them for 4-5 minutes. The slides were washed for 4-5 minutes with distilled water before being dehydrated with 71%, 91%, and 99% ethanol. Finally, slides were stained with propidium (20 μ g/ml), and visualization was performed using an epifluorescence motorized Leica DMLA microscope with automatic digital image scanning software. The procedure was analyzed using a Leica EL6000 light source, a metal lamp halide fitted PL-Fluotar 40 objective, and the image was captured with a PL-Fluotar 60 objective. Images were captured for eight bits in imaging grey level using a CCD DFC-350-FX Leica (scientific grade) [19].

3. Results and Discussion

3.1. Gas chromatography analysis.

Figure S1 depicts the GC-MS chromatogram of an ethyl acetate extract of *P.granatum* seed, and the result shows (Table 1) the presence of twelve main chemicals, one of which is an alkene, 1-Methoxy-2-methyl-3-butene (11.23%), and one of which is alcohol (13.36%). Four of the chemicals in 1-butanol are aldehydes. 3-methyl, 2-hexenal, 2,4-nonadienal, 2,4-decadienal, and 5-hydroxy methyl furfural, totaling 22.97%, one of which is a heterocyclic molecule. Five molecules are fatty acids: tetradecanoic acid, hexadecanoic acid, linoleic acid, octadecanoic acid, and octadecatrienoic acid [20,21]. Among the extract's constituents, fatty acids predominate. The most abundant ingredient in the extract is Octadecatrienoic acid, which accounts for 19.88% of the total extract. It was followed by linoleic acid (16.78%).

Table. 1. GC-MS analysis of phyto compounds obtained in the ethyl acetate extract of *Punica granatum* seed.

S. No	Name	RI	Area %	Formula	Exact mass
1	1-Methoxy-2-methyl-3-butene	624	11.23	C ₆ H ₁₂ O	100.08
2	1-Butanol, 3-methyl-	733	13.36	C ₆ H ₁₀ O ₂	100.16
3	2- hexenal	820	10.58	C ₆ H ₁₂ O ₆	100.13
4	2,4-Nonadienal	854	3.40	C ₉ H ₁₄ O	138.10
5	2,4-Decadienal, (E, E)-	1256	3.34	C ₁₀ H ₁₆ O	152.12
6	5-hydroxy methylfurfural	1267	5.65	C ₆ H ₆ O ₃	126.12
7	Methyl-2- phenylindole	1665	5.86	C ₁₅ H ₁₃ N	207.27
8	Tetradecanoic acid	1730	1.75	C ₁₄ H ₂₈ O ₂	228.34
9	Hexadecanoic acid	1984	1.69	C ₁₆ H ₃₂ O ₂	256.42
10	linoleic acid	2078	16.78	C ₁₈ H ₃₄ O ₂	282.5
11	Octadecanoic acid	2161	3.43	C ₁₈ H ₃₄ O ₂	282.5
12	Octadecatrienoic acid	2207	19.88	C ₁₈ H ₃₀ O ₂	88.11

3.2. Isolation and characterization of PG-1.

Overall, 45 fractions have been collected, and each fraction is monitored by TLC. The 11th fraction from petroleum ether: ethyl acetate (8:2) combinations produces a single spot in TLC. Further, it is taken for spectral analysis, and it is named PG-1. Its UV-vis, FT-IR, ¹³C NMR, and ¹H NMR were used to characterize it and identify its structure. The evaluation of UV-visible spectra revealed an absorption maximum of 250 nm (Figure S2). The FT-IR spectra of PG-1 revealed distinct absorption bands at 1740 cm⁻¹ for the (C=O) Carbonyl group, 1640 cm⁻¹ for the C=C group, and 1100 cm⁻¹ for the C-O group (Figure S3).

The triplet signals at δ 0.88, 0.90, and 0.92 for nine protons in the ¹H NMR spectrum (Figures S4-S6) are attributable to three terminal methyl groups. It was complemented by the presence of the equivalent carbon atoms in the ¹³C -NMR spectra at 13.93, 13.96, and 14.12, respectively [22]. The existence of fatty acid moieties is suggested by the presence of bulky methylene group protons at 1.25 and 1.30. The acylated oxymethylene protons of the glycerol moiety are assigned to the pair of doublets signals at 4.26 and 4.34, but the acylated methylene proton resonated at 5.26, implying that PG-1 is a triglyceride fatty ester. Table S1 shows the ¹³C -NMR data for PG-1 that strongly supports the triglyceride structure.

The presence of numerous unsaturated -CH=CH- bonds is suggested by many signals at δ 5.60 and 6.02 in the ¹H NMR spectra. It was supplemented by its ¹³C-NMR spectra, which showed eight signals ranging from δ 126.10 to 134.52 [23]. The signals at 2.84 in ¹H NMR for two protons are allocated to protons connected to the fatty acids bis-allylic carbon atoms (Figures S7-S9). The allylic protons resonated at 2.07, whereas the protons connected to the fatty acid moiety's -carbon atoms emerged at 2.31. At 1.60, the protons connected to the -carbon atoms appeared.

The two signals one signal with double the intensity corresponds to three carbonyl functions at δ 173.27, 172.86, terminal methyl groups with three signals at δ 14.12, 13.92, and 13.93, a glyceridic primary carbon atom at 62.12 in the ¹³C -NMR spectra (Figure S3), and the terminal methyl groups at δ 0.89 in the ¹H-NMR in the same setting suggests that the three acids may be the same and are esterified in the oxymethylene group of the glycerol moiety. Punic acid appears to be the acid, according to Table S1, and all of the other signals suggest that the molecule is tri-acylated glycerol. The molecule was identified as tri-O-punicyl glycerol according to the above spectrum data (Figure 1). Based on the spectral studies, the structure of the active constituents is drawn and shown in Figure 1. Further, its (PG-1) molecular weight was calculated as 915.42 g/mol, and the molecular formula is C₆₀H₉₈O₆.

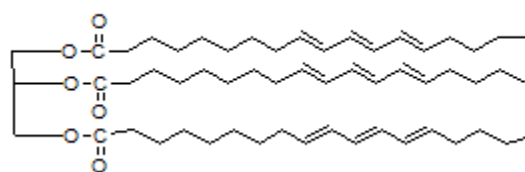


Figure 1. Structure of PG-1.

3.3. *In vitro* antioxidant activity of PG-1.

The free radical scavenging activity of PG-1 was assessed using the DPPH and ABTS assays. The IC₅₀ values of PG-1 in the DPPH assay were found at 26 ±5.7 µg/ml and 24±6.8 µg/ml, respectively, as shown in Figure S6 A. The ABTS assay revealed that the IC₅₀ values for PG-1 and L-ascorbic acid were 26±0.62 µg/ml and 23±0.65µg/ml, respectively (Figure S10)

A, B). In both approaches, the chemical has a comparable scavenging activity to the reference compound [24].

3.4. The antiproliferative ability of PG-1 using the MTT assay.

MTT assay was used to determine PG-1's *in vitro* cytotoxic activity against the A549 lung cancer cell line [14]. The anticancer efficacy revealed that PG-1 had no toxic effects on the fibroblast cell line (3T3), though on the lung cancer cell line, PG-1 inhibited cancer proliferation, and the IC₅₀ value was 25±8.5 µg/ml (Figure 2 A and B; Figure 3) compared to the standard doxorubicin (IC₅₀ was 22±7.4 µg/ml) [10,25].

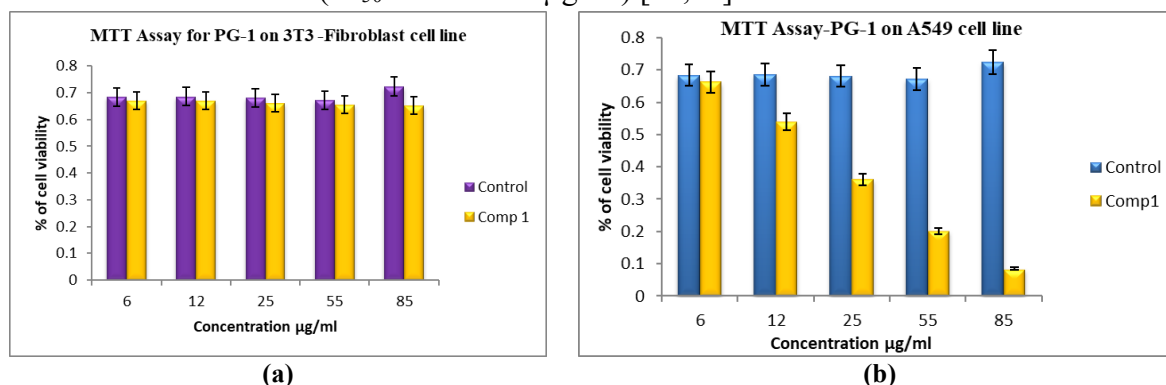


Figure 2. (a) Effect of different concentrations of PG-1 on 3T3 (fibroblast cell line); (b) on A549 (lung cancer cell line).

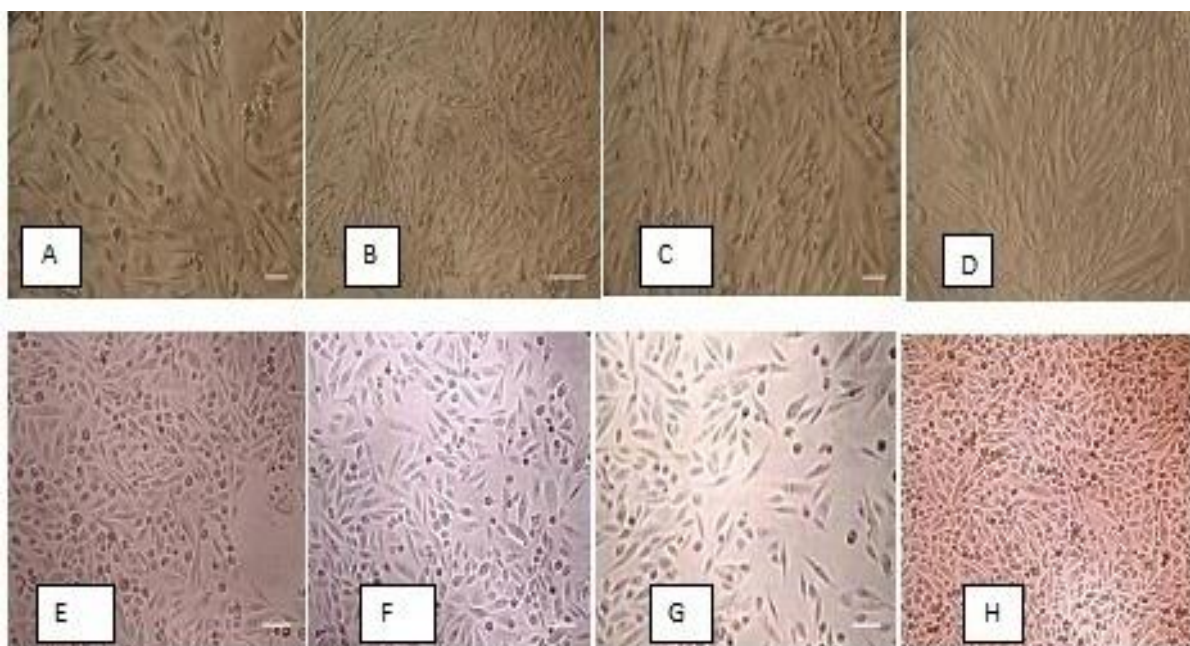


Figure 3. (A-D) represents the morphological changes of normal fibroblast cell lines upon PG-1 treatment (A-High dose; B-Low dose; C-Medium dosage; D-Control); (E-H) represents the morphological changes of A549 cell lines with PG-1 treatment at different concentrations (E-6µg/ml; F-12µg/ml; G-25µg/ml; H-Control).

3.5. Genotoxic activity of PG-1 on lung cancer cell line by Comet assay.

The effects of PG-1 on lung cancer cell lines were studied using the comet assay [26], and DNA damage was seen in the A549 cell line at a dose of 250 µg/ml. An increase in the percentage of tail DNA damage was reported using open comet software as compared to the control. The greater the damage, the longer the tail, and PG-1 proved capable of eliminating

tumor DNA in a dose-dependent manner (Figure.4). As a result, it indicated that PG-1 functions as an effective anticancer candidate [27].

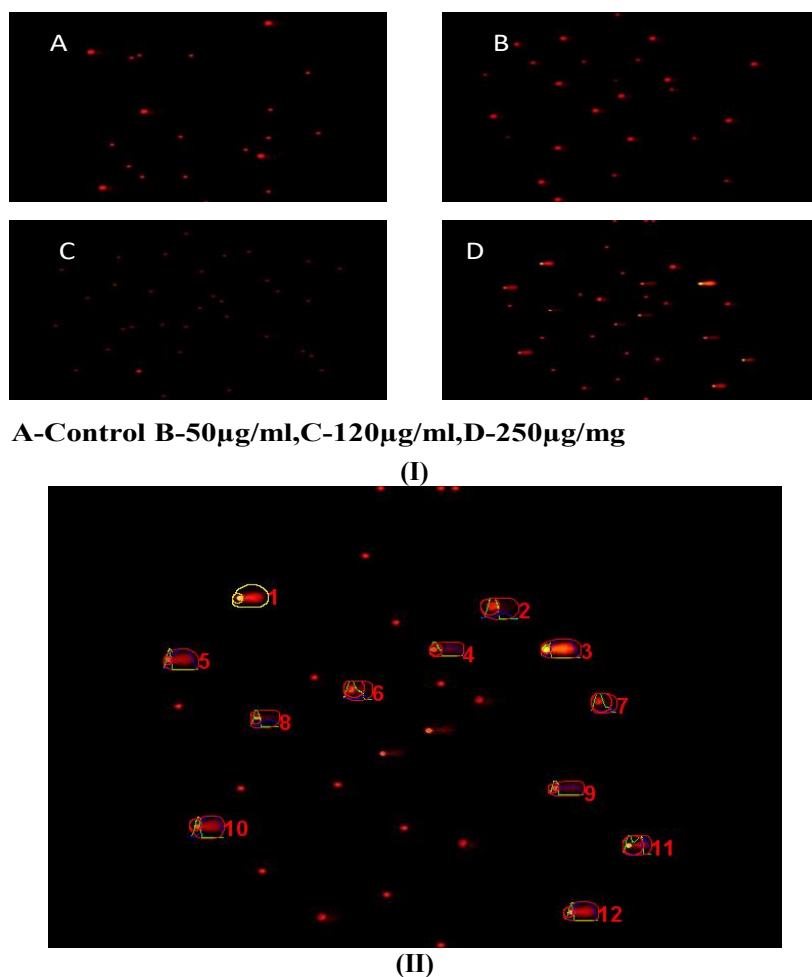


Figure 4. (I) Gene toxic effect of PG-1 on the A549 cell line at various concentrations; (II) DNA-damaging effects of PG-1 on the A549 cell line.

3.6. Cell cycle Analysis of PG-1 on lung cancer cell line by flow cytometry.

The cell cycle distribution analysis was performed on the PG-1 utilizing a flow cytometer to determine its influence on the A549 cell line. At a 12 µg/ml concentration, the cells were arrested at the G₀ phase after 48 hours as compared to the control [28,29]. The A549 cell line was halted in the G₀ cell cycle phase after being treated with PG-1 (Figure 5). Due to the strong proliferative status of this cell line, the majority of cells (60%) were accumulated at time 0. Control cells that were not treated showed a normal pattern for constantly growing cells. As a result, PG-1 effectively inhibits the cell cycle development of the cells. These findings need to be evaluated in clinical trials for lung cancer [30].

It may be counterintuitive to suggest triglyceride as a lead phytochemical for cancer therapy. Higher levels of triglycerides in the blood may be associated with cancer initiation and development because they may serve as a rich source of energy for developing tumors [31,32]. Nonetheless, high levels of circulating triglycerides correspond with a better prognosis in certain malignancies, such as breast cancer [33] and prostate cancer [31]. Furthermore, certain lipids, such as linoleic acid, have been indicated as natural anticarcinogenic molecules in a few studies [34-35].

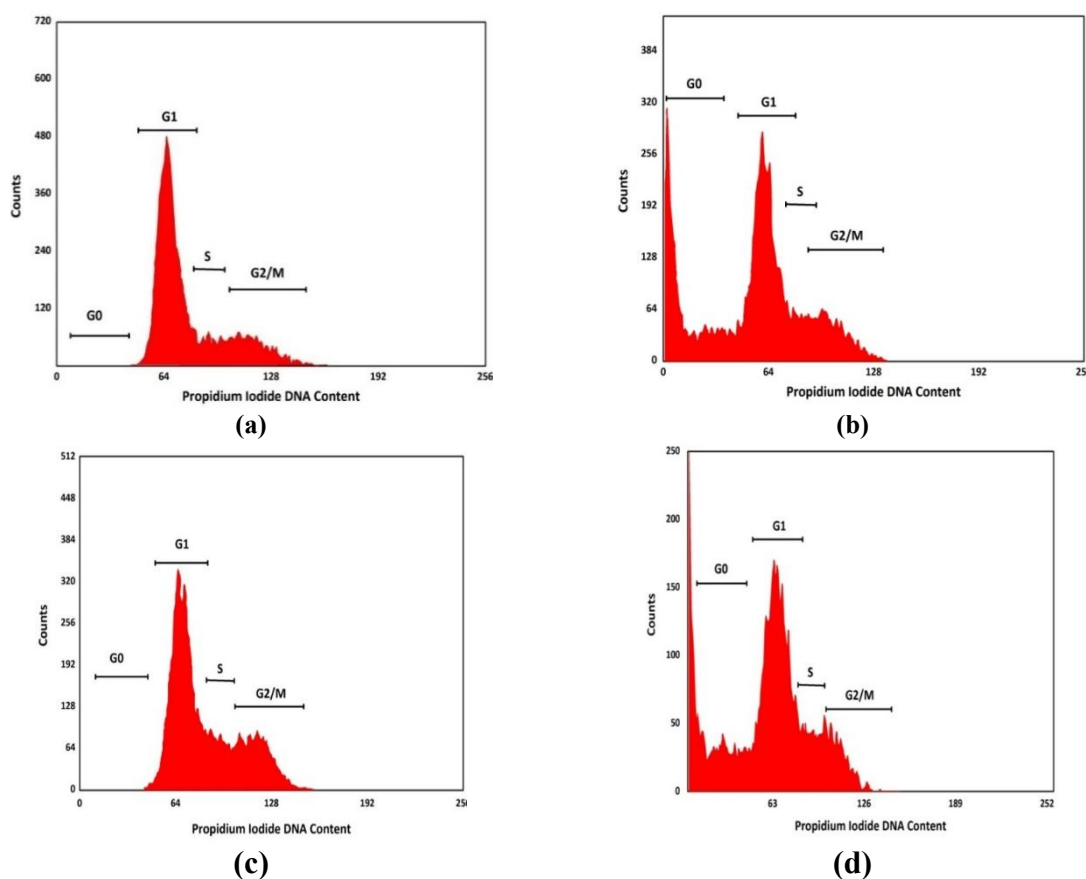


Figure 5. Cell cycle analysis of PG-1 on the A549 cell line by flow cytometry. A549 cells were treated with PG-1 (a) 6 μ g/ml; (b) 12 μ g/ml; (c)Control; (d) IC₅₀ concentration for 24 hrs.

Furthermore, lung cancer is one of the most prevalent causes of death in both men and women around the world, and cigarette smoking is a key cause of the condition since cigarette smoke promotes a boost in oxidative stress and DNA damage. In the current study, because PG-1 has both anticytotoxic and antioxidant activity, it can be used as a natural plant-based therapy with fewer adverse effects against the lung cancer cell line (A549). It can also be utilized as an adjuvant in the treatment of lung cancer in patients who are receiving methotrexate.

4. Conclusion

Nowadays, natural-based medicine with fewer side effects is in demand in the pharmaceutical industry due to the fact that modern chemotherapies produce numerous side effects. In view of that, we have attempted to find a natural lead molecule from a natural plant source. The active tri-O-punicyl glycerol (PG-1) isolated from ethyl acetate extract of *Punica granatum* seed has higher cytotoxic effects on the lung cancer cell line (A549) while having no negative effects on the fibroblast cell line. The comet assay indicated DNA damage, and cell cycle studies against the A549 cell line demonstrated that PG-1 inhibited cell growth in the G0 phase. PG-1 also has strong antioxidant properties. This can be used as a lead phytochemical from a natural plant source, *Punica granatum* seeds, as a natural therapy with fewer side effects against the lung cancer cell line (A549). Further, this finding needs to be evaluated in clinical trials for natural-based drug inventions for the treatment of lung cancer.

Author Contributions

Conceptualization, S.A.Y.; data curation, S.A.Y. and R.S.; writing—review and editing, S.A.Y.; supervision, S.A.Y.; funding acquisition, S.A.Y.; methodology, S.S.N.; formal analysis, S.S.N.; writing—original draft preparation, S.S.N. All authors have read and agreed to the published version of the manuscript

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

Funding

The authors are pleased to acknowledge DST-SHRI (File No. DST/TDT/SHRI/2022/70) and DST-FIST (File No. SR/FST/LS-1/2018/187) and Karpagam Academy of Higher Education for their support in carrying out this work.

Acknowledgments

The support provided by the Department of Biotechnology, Karpagam Academy of Higher Education, Coimbatore, Tamil Nadu, India, is gratefully acknowledged.

Conflict of Interest

None.

References

1. Liu, C.; Wei, S.; Liu, L. ASO Author Reflections: Individualized Mediastinal Lymph Node Dissection for Lung Cancer: Do Not Neglect Station 3A Lymph Node. *Ann. Surg. Oncol.* **2020**, *27*, 846-847, <https://doi.org/10.1245/s10434-020-08876-x>.
2. Green, M.R.; Manikhas, G.M.; Orlov, S.; Afanasyev, B.; Makhson, A.M.; Bhar, P.; Hawkins, M.J. Abraxane®, a novel Cremophor®-free, albumin-bound particle form of paclitaxel for the treatment of advanced non-small-cell lung cancer. *Ann. Oncol.* **2006**, *17*, 1263-1268, <https://doi.org/10.1093/annonc/mdl104>.
3. James, A.; Akash, K.; Sharma, A.; Bhattacharyya, S.; Sriamornsak, P.; Nagraik, R.; Kumar, D. Himalayan flora: targeting various molecular pathways in lung cancer. *Med. Oncol.* **2023**, *40*, 314, <https://doi.org/10.1007/s12032-023-02171-x>.
4. Asadi-Samani, M.; Rafieian-Kopaei, M.; Lorigooini, Z.; Shirzad, H. A screening of growth inhibitory activity of Iranian medicinal plants on prostate cancer cell lines. *BioMedicine* **2018**, *8*, 8, <https://doi.org/10.1051/bmdcn/2018080208>.
5. Valero-Mendoza, A.G.; Meléndez-Rentería, N.P.; Chávez-González, M.L.; Flores-Gallegos, A.C.; Wong-Paz, J.E.; Govea-Salas, M.; Zugasti-Cruz, A.; Ascacio-Valdés, J.A. The whole pomegranate (*Punica*

- granatum*. L), biological properties and important findings: A review. *Food Chem. Adv.* **2023**, *2*, 100153, <https://doi.org/10.1016/j.focha.2022.100153>.
6. Xu, Y.-Y.; Wang, W.-W.; Huang, J.; Zhu, W.-G. Ellagic acid induces esophageal squamous cell carcinoma cell apoptosis by modulating SHP-1/STAT3 signaling. *Kaohsiung J. Med. Sci.* **2020**, *36*, 699-704, <https://doi.org/10.1002/kjm2.12224>.
 7. Tashkandi, H.M. Therapeutic Potential of Pomegranate (*Punica granatum* Linn.) against Breast Cancer. *Indian J. Pharm. Sci.* **2023**, *85*, <http://dx.doi.org/10.36468/pharmaceutical-sciences.spl.626>.
 8. Pantiora, P.D.; Balaouras, A.I.; Mina, I.K.; Freris, C.I.; Pappas, A.C.; Danezis, G.P.; Zoidis, E.; Georgiou, C.A. The Therapeutic Alliance between Pomegranate and Health Emphasizing on Anticancer Properties. *Antioxidants* **2023**, *12*, 187, <https://doi.org/10.3390/antiox12010187>.
 9. Mukherjee, S.; Ghosh, S.; Choudhury, S.; Adhikary, A.; Manna, K.; Dey, S.; Sa, G.; Das, T.; Chattopadhyay, S. Pomegranate reverses methotrexate-induced oxidative stress and apoptosis in hepatocytes by modulating Nrf2-NF-κB pathways. *The Journal of Nutritional Biochemistry* **2013**, *24*, 2040-2050, <https://doi.org/10.1016/j.jnutbio.2013.07.005>.
 10. Nelson, S.S.; Yadav, S.A.; Surendren, L.K. Evaluation of in vitro anticancer potential in *Punica granatum*, *Psidium guajava*, and *Vitis vinifera* seed extracts. *Int. J. Res. Pharm. Sci.* **2019**, *10*, 165-169.
 11. Gobinath, A.; Yadav, S.A. Comparative Evaluation of the Essential Oil Content of Asteraceae Family Medicinal Plants. *Curr. Anal. Chem.* **2024**, <https://doi.org/10.2174/0115734110316947240813054000>.
 12. Shimizu, G.D.; Vidal, T.C.M.; Junior, W.A.R.; Ribeiro, L.T.M.; Castilho, Í.M.; Gonçalves, L.S.A.; Silva, J.B. Antioxidant activity and chemical composition from different parts of pomegranate (*Punica granatum* L.) cultivars. *Sci. Elec. Arch.* **2023**, *16*, <https://doi.org/10.36560/16420231675>.
 13. Kumar, S.; Singh, V.; Johar, V.; Varma, S.; Thakur, R. Antioxidant Activity, Antibacterial Activity and Phenolic Composition of Pomegranate Fruit: A Review. *J. Food Chem. Nanotechnol.* **2023**, *9*, S588-S593, <https://doi.org/10.17756/jfcn.2023-s1-074>.
 14. Niu, J.; Jia, X.; Yang, N.; Ran, Y.; Wu, X.; Ding, F.; Tang, D.; Tian, M. Phytochemical analysis and anticancer effect of *Camellia oleifera* bud ethanol extract in non-small cell lung cancer A549 cells. *Front. Pharmacol.* **2024**, *15*, 1359632, <https://doi.org/10.3389/fphar.2024.1359632>.
 15. Bandpi, A.M.; Eslami, A.; Ghaderpoori, M.; Shahsavani, A.; Mohammadi, A.A.; Morovati, M.; Gholampoor, Y.; Jeihooni, A.K.; Ghaderpoury, A.; Parseh, I.; Alinejad, A.; Sadeghi, S. Characteristics and sources of water-soluble ionic associated with PM_{2.5} particles and cytotoxicity effects using MTT assay in Tehran, Iran. *Urban Clim.* **2020**, *32*, 100612, <https://doi.org/10.1016/j.uclim.2020.100612>.
 16. Manoharan, S.P.; Yadav, S.A.; Pandiyan, B.; Suvathika, G. *Tragia plukenetii*-Assisted Omega-Decenol as Potential Anticancer Agent: its Isolation, Characterization, and Validation. *Appl. Biochem. Biotechnol.* **2023**, *195*, 1699-1722, <https://doi.org/10.1007/s12010-022-04221-y>.
 17. Hongal, A.M.; Shettar, A.K.; Hoskeri, J.H.; Vedamurthy, A.B. Silver nanoparticles mediated apoptosis and cell cycle arrest in lung cancer A549. *3 Biotech* **2024**, *14*, 238, <https://doi.org/10.1007/s13205-024-04064-w>.
 18. Elhaddad, N.S.; Khatab, H.A.; Habib, I.H.I. In-vitro Repair Ability of DNA Damage by *Anethum graveolens* L. Aqueous Extract Using Comet Assay. *Asian J. Res. Biochem.* **2024**, *14*, 43-54, <https://doi.org/10.9734/ajrb/2024/v14i5310>.
 19. Vijayakumar, N.; Alagar, S.; Madanagopal, N. Biocontrol potential of fungal chitinase from high yielding *trichoderma viride* against *corcyra cephalonica* (stainton). *Int. J. Pharm. Bio. Sci.* **2017**, *8*, 447-452, <https://doi.org/10.22376/ijpbs.2017.8.2.b447-452>.
 20. Ibrahim, H.; Uttu, A.J.; Sallau, M.S.; Iyun, O.R.A. Gas chromatography–mass spectrometry (GC–MS) analysis of ethyl acetate root bark extract of *Strychnos innocua* (Delile). *Beni-Suef Univ. J. Basic Appl. Sci.* **2021**, *10*, 65, <https://doi.org/10.1186/s43088-021-00156-1>.
 21. Pinto, J.; Oliveira, A.S.; Lopes, P.; Roseira, I.; Cabral, M.; Bastos, M.d.L.; Guedes de Pinho, P. Characterization of chemical compounds susceptible to be extracted from cork by the wine using GC-MS and ¹H NMR metabolomic approaches. *Food Chem.* **2019**, *271*, 639-649, <https://doi.org/10.1016/j.foodchem.2018.07.222>.
 22. Sunday, A.S.; Abosedede, F.T.; Daniel, E.E.; Yetunde, A.A.; Adenike, S.R.; Oluwasanmi, L.I. Triglyceride composition of almond seed oil (*Terminalia catappa*) grown in Nigeria using GC-MS and ¹H-NMR spectroscopy. *Eur. J. Nutr. Food Saf.* **2021**, *13*, 41-49, <https://doi.org/10.9734/ejnf/2021/v13i430402>.

23. Tengku-Rozaina, T.M.; Birch, E.J. Positional distribution of fatty acids on hoki and tuna oil triglycerides by pancreatic lipase and ¹³C NMR analysis. *Eur. J. Lipid Sci. Technol.* **2014**, *116*, 272-281, <https://doi.org/10.1002/ejlt.201300357>.
24. Mandal, A.; Bishayee, A. Mechanism of Breast Cancer Preventive Action of Pomegranate: Disruption of Estrogen Receptor and Wnt/β-Catenin Signaling Pathways. *Molecules* **2015**, *20*, 22315-22328, <https://doi.org/10.3390/molecules201219853>.
25. Khayam, A.U.; Patel, H.; Faiola, N.A.; Figueroa Milla, A.E.; Dilshad, E.; Mirza, B.; Huang, Y.; Sheikh, M.S. Quinovic acid purified from medicinal plant *Fagonia indica* mediates anticancer effects via death receptor 5. *Mol. Cell. Biochem.* **2020**, *474*, 159-169, <https://doi.org/10.1007/s11010-020-03841-4>.
26. Mei, J.; Liu, S.; Li, Z.; Gui, J.-F. Mtmr8 is essential for vasculature development in zebrafish embryos. *BMC Dev. Biol.* **2010**, *10*, 96, <https://doi.org/10.1186/1471-213X-10-96>.
27. Ramos, A.A.; Castro-Carvalho, B.; Prata-Sena, M.; Dethoup, T.; Buttachon, S.; Kijjoa, A.; Rocha, E. Crude Extracts of Marine-derived and Soil Fungi of the Genus *Neosartorya* Exhibit Selective Anticancer Activity by Inducing Cell Death in Colon, Breast and Skin Cancer Cell Lines. *Pharmacogn. Res.* **2016**, *8*, 8-15, <https://doi.org/10.4103/0974-8490.171105>.
28. Modem, S.; DiCarlo, S.E.; Reddy, T.R. Fresh Garlic Extract Induces Growth Arrest and Morphological Differentiation of MCF7 Breast Cancer Cells. *Genes Cancer* **2012**, *3*, 177-186, <https://doi.org/10.1177/1947601912458581>.
29. Li, Y.; Yang, F.; Zheng, W.; Hu, M.; Wang, J.; Ma, S.; Deng, Y.; Luo, Y.; Ye, T.; Yin, W. *Punica granatum* (pomegranate) leaves extract induces apoptosis through mitochondrial intrinsic pathway and inhibits migration and invasion in non-small cell lung cancer in vitro. *Biomed. Pharmacother.* **2016**, *80*, 227-235, <https://doi.org/10.1016/j.biopha.2016.03.023>.
30. Siddiqui, S.; Arshad, M. Osteogenic potential of *punica granatum* through matrix mineralization, cell cycle progression and runx2 gene expression in primary rat osteoblasts. *DARU J. Pharm. Sci.* **2014**, *22*, 72, <https://doi.org/10.1186/s40199-014-0072-7>.
31. Ulmer, H.; Borena, W.; Rapp, K.; Klenk, J.; Strasak, A.; Diem, G.; Concini, H.; Nagel, G.; Vhm; Group, P.P.S. Serum triglyceride concentrations and cancer risk in a large cohort study in Austria. *Br. J. Cancer* **2009**, *101*, 1202-1206, <https://doi.org/10.1038/sj.bjc.6605264>.
32. Shen, Y.; Wang, C.; Ren, Y.; Ye, J. A comprehensive look at the role of hyperlipidemia in promoting colorectal cancer liver metastasis. *J. Cancer* **2018**, *9*, 2981-2986, <https://doi.org/10.7150/jca.25640>.
33. Ni, H.; Liu, H.; Gao, R. Serum Lipids and Breast Cancer Risk: A Meta-Analysis of Prospective Cohort Studies. *PLoS ONE* **2015**, *10*, e0142669, <https://doi.org/10.1371/journal.pone.0142669>.
34. Dachev M, Bryndová J, Jakubek M, Moučka Z, Urban M. The effects of conjugated linoleic acids on cancer. Processes. 2021 Mar 3;9(3):454.. <https://doi.org/10.3390/pr9030454>.
35. Vilariño, M.; García-Sanmartín, J.; Ochoa-Callejero, L.; López-Rodríguez, A.; Blanco-Urgoiti, J.; Martínez, A. Macrocybin, a Natural Mushroom Triglyceride, Reduces Tumor Growth In Vitro and In Vivo through Caveolin-Mediated Interference with the Actin Cytoskeleton. *Molecules* **2020**, *25*, 6010, <https://doi.org/10.1101/2020.12.10.418863>.

Publisher's Note & Disclaimer

The statements, opinions, and data presented in this publication are solely those of the individual author(s) and contributor(s) and do not necessarily reflect the views of the publisher and/or the editor(s). The publisher and/or the editor(s) disclaim any responsibility for the accuracy, completeness, or reliability of the content. Neither the publisher nor the editor(s) assume any legal liability for any errors, omissions, or consequences arising from the use of the information presented in this publication. Furthermore, the publisher and/or the editor(s) disclaim any liability for any injury, damage, or loss to persons or property that may result from the use of any ideas, methods, instructions, or products mentioned in the content. Readers are encouraged to independently verify any information before relying on it, and the publisher assumes no responsibility for any consequences arising from the use of materials contained in this publication.