



Research Article

Anti-proliferative effects of leaf extract of *Petroselinum sativum* on human lung and colon cancer cells: Mitochondrial-mediated apoptosis

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ABSTRACT

Plant-derived natural products have attracted considerable attention for their potential in treating human diseases, particularly cancer. This study aimed to evaluate whether the ethanolic *Petroselinum sativum* ethanolic extract (PS-Et) can inhibit the growth of human lung carcinoma (A-549) and human colon adenocarcinoma (CaCo-2) cell lines. Leaves were extracted with 80% ethanol and tested for effects on cell viability, morphology, reactive oxygen species (ROS) production, mitochondrial membrane potential (MMP) level, caspase enzymes, and apoptosis-related genes. In both cell lines, PS-Et significantly reduced cell viability in a dose-dependent manner, with an IC₅₀ value of 17.6 µg/mL and 21.2 µg/mL for A-549 and CaCo-2 cells, respectively. Further assays on morphology, ROS generation, mitochondrial membrane potential, caspase enzymes, and real-time qPCR were performed in A-549 cells using cytotoxic doses of PS-Et. PS-Et markedly reduced the growth of A-549 and CaCo-2 cells and induced notable changes in their morphology. Furthermore, PS-Et dose-dependently increased ROS generation, disrupted mitochondrial function, and activated caspase enzymes in A-549 cells. Mitochondrial-mediated apoptosis was evident, as shown by the increased expression of p53, Bax, caspase-3, and caspase-9, along with decreased expression of the anti-apoptotic gene, Bcl-2. Collectively, PS-Et exerts a potent anticancer effect by promoting mitochondrial dysfunction, oxidative stress, and the intrinsic apoptotic pathway in A-549 cells. These findings suggest that PS-Et extract could serve as a potential therapeutic candidate for combating cancer.

1. Introduction

Cancer is one of the most critical health challenges and remains a leading cause of death globally. With the acceleration of modern technological advancements, the incidence of life-threatening genetic disorders such as cancer has markedly increased (Wu *et al.*, 2024). Persistent exposure to environmental toxicants can disrupt normal cellular functions, leading to genetic alterations and damage (Chen *et al.*, 2025). Moreover, inadequate dietary intake of fruits and vegetables, along with detrimental lifestyle habits such as smoking and alcohol consumption, further elevate the risk of developing cancer (Marino *et al.*, 2024). A major challenge in the treatment of various cancers is the severe toxicity associated with most chemotherapeutic agents, along with the frequent development of drug resistance (Khan *et al.*, 2024). Furthermore, conventional cytotoxic therapies extensively target rapidly dividing cells, affecting not only malignant but also normal proliferating cells. This non-selectivity often results in substantial morbidity while providing only a limited therapeutic advantage to patients (Anand *et al.*, 2023). Natural products are often preferred by patients due to their comparatively lower side effects and more balanced chemical nature compared to synthetic drugs (Aware *et al.*, 2022). Plants have long served and continue to serve as a vital source

of new anticancer agents (Asma *et al.*, 2022). A significant number of anticancer drugs currently in clinical use are either natural products or derived from natural sources. Studies have shown that 68% of 136 small-molecule anticancer drugs approved by the US-FDA between 1940 and 2014 originated from natural compounds or were developed based on plant-derived molecules (Naeem *et al.*, 2022). In other words, botanicals and traditional medicines hold great promise as anticancer agents, as they contain diverse bioactive compounds with the ability to target and eliminate proliferating cells rapidly (Banerjee *et al.*, 2023). Currently, there is a growing interest in the use of herbal medicine for cancer therapy, owing to its relatively low toxicity toward normal tissues and its selective action against cancer cells. Herbal remedies are progressively explored as complementary or alternative treatment options, with natural compounds demonstrating the ability to reduce inflammation and overcome treatment (Jenča *et al.*, 2024). *Petroselinum sativum*, commonly known as Parsley, is a biennial aromatic plant from the *Apiaceae* family. It is widely distributed across various regions, including Africa, the Mediterranean, the Middle East, India, and China (Mahdi *et al.*, 2024). *P. sativum* is rich in bioactive constituents, including volatile terpenes and terpenoids in its essential oils, as well as phenolic compounds in its extracts. Traditionally, it has been employed as a natural remedy for conditions such as urolithiasis, indigestion, and

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as a tonic for the liver, stomach, and urinary system (Bahramsoltani et al., 2024). Pharmacological studies also approve numerous biological activities of Parsley, including antidiabetic (Bolkent et al., 2004), antimicrobial (Wahba et al., 2010), anticancer (Farshori et al., 2013), hepatoprotective (Al-Oqail et al., 2020), antioxidant, and anti-inflammatory (Wang et al., 2025) properties. Hence, in this study, we aimed to investigate the *in vitro* anti-proliferative effects of *Petroselinum sativum* ethanolic extract (PS-Et) on human colon adenocarcinoma (Caco-2) and lung carcinoma (A-549) cell lines. Additionally, we evaluated the impact of PS-Et on cell morphology, reactive oxygen species (ROS) production, mitochondrial membrane potential (MMP) level, caspase enzyme activities, and apoptosis.

2. Materials and Methods

2.1 Chemicals and consumables

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, neutral red, dichlorodihydrofluorescein diacetate (DCFH-DA), Rh-123 dye, along with other chemicals, were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Culture media and growth supplements were sourced from Gibco-Life Technologies Co. (USA).

2.2 Plant material preparation

Fresh leaves of *Petroselinum sativum* were collected from the Riyadh region of Saudi Arabia. The leaves were washed with distilled water and air-dried. Once dried, they were ground into a fine powder using a laboratory grinder and then soaked in ethanol for 24 h at room temperature. The resulting extract was filtered, and the solvent was evaporated under reduced pressure. The dried *Petroselinum sativum* ethanolic extract (PS-Et) was stored in aliquots at 4°C until further use. PS-Et was dissolved in Dimethyl sulfoxide (DMSO) for bioassays.

2.3 Cell culture

Human colorectal adenocarcinoma (Caco-2) and human lung carcinoma (A-549) cell lines were procured from ATCC (USA). Cells were cultured in dulbecco's modified eagle's medium (DMEM) medium supplemented with 100 µg/mL streptomycin, 100 U/mL penicillin, and 10% heat-inactivated fetal bovine serum. Cultures were maintained at 37°C in a humidified incubator with 5% CO₂.

2.4 MTT assay

The MTT colorimetric assay, as described by Siddiqui et al. (2008), was employed to evaluate the inhibitory effects of the ethanolic extract of PS-Et on Caco-2 and A-549 cell lines. Actively proliferating cells were harvested using 0.25% trypsin-EDTA and seeded into 96-well plates at a density of 10,000 cells per well. After 24 h of incubation, cells were treated with varying concentrations (1.56-100 µg/mL) of PS-Et extract for 24 h. Following treatment, 10 µL of MTT (5 mg/mL) was added to wells and incubated further for 4 h. Then, the supernatant was removed, and the developed formazan was dissolved in 200 µL of DMSO. The absorbance of the dissolved formazan solution was determined at 550 nm using a microplate reader.

2.5 NRU assay

Following treatment as described above, cells were incubated with 0.5% neutral red (NR) dye suction for 3 h at 37°C, in the incubator. Then the unbound dye was washed out with washing solution (1% CaCl₂ and 0.5% formaldehyde). The bound NR dye was then solubilized using a 50% ethanol and 1% acetic acid solution, and absorbance was measured at 550 nm with a microplate reader (Multiskan EX, Thermo Scientific, China) (Siddiqui et al. 2008).

2.6 Cell Morphology

To assess the effect of PS-Et on cell morphology, 1 × 10⁴ cells were seeded in 96-well plates and treated with PS-Et at 25, 50, and 100 µg/mL for 24 h. Morphological changes were examined at 20×

magnification using a phase-contrast inverted microscope equipped with a digital camera (Olympus CKX41, Japan).

2.7 ROS measurement

To measure ROS production, (DCFH-DA; Sigma, USA) was employed. DCFH-DA passively enters cells and reacts with ROS to generate dichlorofluorescein (DCF), a highly fluorescent compound. Cells were initially plated at a density of 2 × 10⁴ cells per well in a 24-well plate, incubated with PS-Et at 12.5, 25, and 50 µg/mL for 24 h. Following two washes with Phosphate-Buffered Saline (PBS), the cells were stained with 20 µM of DCFH-DA and incubated for 60 min. After rinsing with PBS, fluorescence intensity was measured relative to control cells. Images of the treated cells were captured using a fluorescent microscope at 20× magnification.

2.8 Mitochondrial membrane potential analysis

MMP was evaluated using rhodamine-123 (Rh-123; Sigma, USA). In brief, cells were seeded at 2 × 10⁴ cells per well in 24-well plates and treated with PS-Et at 12.5, 25, and 50 µg/mL for 24 h. After two PBS washes, cells were stained with Rh-123 (10 µg/mL) for 1 h, rinsed, and fluorescence intensity was measured relative to untreated controls. Images were captured using a fluorescence microscope (Olympus CKX41, Japan) at 20× magnification.

2.9 Caspase-3 and -9 enzyme activity

Caspase-3 and -9 activity was measured in cells treated for 24 h with 12.5, 25, and 50 µg/mL concentrations of PS-Et, using a human caspase-3 and -9 ELISA kit (Biovision), following the manufacturer's protocol.

2.10 Real-time PCR (RT-PCR) analysis

Total RNA was isolated from A-549 cells using an RNA extraction kit (Qiagen) following the manufacturer's protocol. One microgram of RNA from each sample was reverse transcribed into cDNA with the MLV reverse transcriptase kit (GE Healthcare, UK). Quantitative PCR was conducted on a Roche® LightCycler® 480 system using SYBR Green Master Mix (Roche Diagnostics). Primer sequences and cycling conditions used are listed in our previous publication (Al-Oqail et al., 2017). Relative mRNA expression levels were calculated using the Ct method, with GAPDH serving as the internal control.

2.11 Statistical analysis

Statistical differences between the treated and untreated groups were analyzed using one-way ANOVA applying Dunnett's test. A *p*-value below 0.05 was considered indicative of statistical significance.

3. Results

3.1 Cytotoxicity of PS-Et by MTT assay

The anti-proliferative effect of PS-Et was evaluated using the MTT assay on A-549 and Caco-2 cell lines. As presented in Fig. 1, the PS-Et extract exhibited significant cytotoxicity across both cell lines. MTT assay results showed a concentration-dependent reduction in cell viability after 24 h of treatment. In A-549 cells, viability decreased to 87%, 69%, 29%, 25%, and 17% at concentrations of 6.25, 12.5, 25, 50, and 100 µg/mL, respectively (Fig. 1a). Similarly, Caco-2 cells exhibited viabilities of 78%, 41%, 30%, and 23% at the same concentrations (Fig. 1b). A-549 cells were the most sensitive, with an IC₅₀ of 17.6 µg/mL, compared to Caco-2, with an IC₅₀ of 21.2 µg/mL.

3.2 Cytotoxicity of PS-Et by NRU assay

Consistent results were also obtained with the NRU assay. Exposure to PS-Et led to a dose-dependent reduction in the viability of both cancer cell lines. In A-549 cells, viability decreased to 91%, 65%, 31%, 26%, and 19% at 6.25, 12.5, 25, 50, and 100 µg/mL PS-Et, respectively,

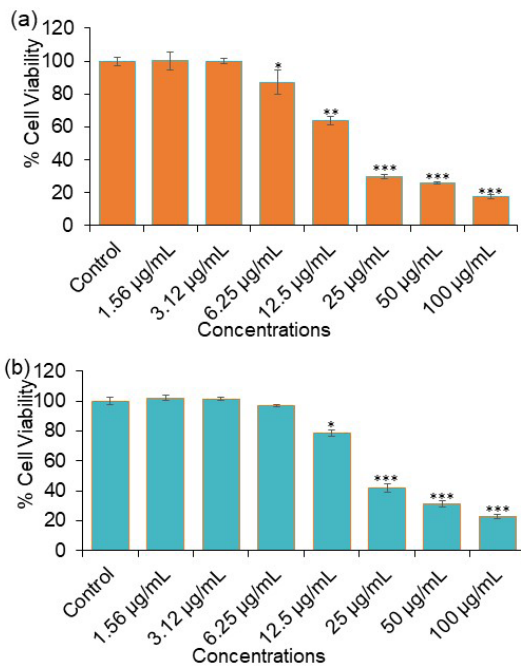


Fig. 1. Percentage of cell viability as measured by MTT assay in (a) A-549 and (b) Caco-2 cells after 24 h exposure of PS-Et at different concentrations. Data are presented as mean \pm SD from at least three independent experiments. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ indicate significant differences compared to the respective control.

after 24 h (Fig. 2a). Similarly, Caco-2 cells exhibited viabilities of 75%, 43%, 28%, and 25% at 12.5, 25, 50, and 100 $\mu\text{g/mL}$ PS-Et (Fig. 2b). Lower concentrations i.e. 1.56 and 3.12 $\mu\text{g/mL}$ of PS-Et did not produce any toxicity to A-549 and Caco-2 cells. Similar to MTT results, A-549 cells were the most sensitive, with an IC_{50} of 18.1 $\mu\text{g/mL}$, compared to Caco-2, with an IC_{50} of 21.9 $\mu\text{g/mL}$. Based on the cytotoxicity results, further studies were conducted on A-549 cells.

3.3 PS-Et induces morphological changes in A-549 and Caco-2 cells

After 24 h of treatment with PS-Et (25, 50, and 100 $\mu\text{g/mL}$), morphological alterations were observed in A-549 and Caco-2 cells. Compared with untreated controls, the treated cells showed reduced adhesion and spreading, with irregular shapes. As shown in Fig. 3, many cells appeared shrunken and rounded, and some had detached from the culture surface.

3.4 Effect of PS-Et on ROS production

Compared with the control, ROS generation was significantly elevated in the groups treated with PS-Et extract, showing a clear dose-dependent pattern (Fig. 4). PS-Et concentrations between 12.5 and 50 $\mu\text{g/mL}$ markedly influenced ROS production by 121% to 180%.

3.5 Effect of PS-Et on MMP level

As shown in Fig. 5, the effect of PS-Et on MMP was assessed by Rh-123 dye after 24 h treatment of A-549 cells. The results demonstrated a dose-dependent loss of 21%, 35%, and 47% in MMP level at 12.5, 25, and 50 $\mu\text{g/mL}$, respectively.

3.6 Effect of PS-Et on caspase-3 and -9 enzymes

To evaluate the effect of PS-Et extract on the proteolytic phase of apoptosis, caspase-3 and -9 activity was measured in the control and treated cells. The extract induced a significant increase in caspase-3 and -9 levels in A-549 cells, with elevations of 113%, 133%, and 162% at 12.5, 25, and 50 $\mu\text{g/mL}$, respectively (Fig. 6a). In contrast,

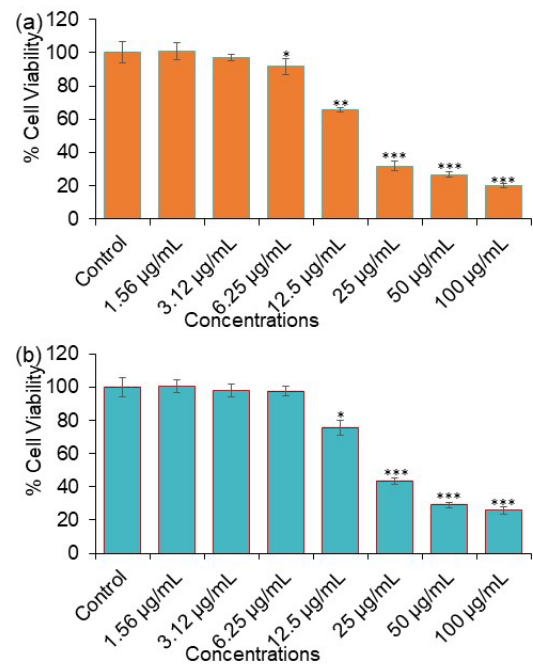


Fig. 2. Percentage of cell viability as measured by neutral red uptake assay in (a) A-549 and (b) Caco-2 cells after 24 h of exposure to PS-Et at different concentrations. Data are presented as mean \pm SD from at least three independent experiments. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ indicate significant differences compared to the respective control.

caspase-9 activity was elevated to 121%, 145%, and 173% at the same concentrations of PS-Et (Fig. 6b).

3.7 Effect of PS-Et on gene expression

A-549 cells were evaluated by analyzing the mRNA expression of p53, Bax, caspase-3, caspase-9, and Bcl-2 genes. Treatment with PS-Et, in comparison to untreated controls, led to a significant upregulation of the pro-apoptotic genes (p53, Bax, caspase-3, and caspase-9) and a marked downregulation of the anti-apoptotic gene Bcl2 (Fig. 7).

4. Discussion

Petroselinum sativum is recognized as a dependable source of herbal medicinal products with a wide range of biological activities (Bahramsoltani et al., 2024). Hence, this research aimed to explore the cytotoxic effects of *Petroselinum sativum* ethanolic extract (PS-Et) on cultured human lung (A-549) and colon (Caco-2) cancer cells *in vitro*. The findings revealed that PS-Et exerts cytotoxic effects on cancer cells. Additionally, the results indicated that cell death occurred through apoptosis, initiated by ROS-mediated mitochondrial pathways, as demonstrated by the caspase enzyme activities and mitochondrial membrane dysfunction.

To strengthen the reliability of our data, we first assessed the cytotoxicity of PS-Et using two different assays: MTT and NRU. The MTT assay depends on the metabolic adaptation of the soluble MTT salt into an insoluble formazan product. This reaction, which imitates the activity of mitochondrial dehydrogenase enzymes in living cells, serves as an indicator of cell viability (Mosmann, 1983). The NRU assay bank on the ability of viable cells to fascinate and hold neutral red, a weak cationic dye that passes in cells through non-ionic diffusion and accumulates within lysosomes (Borenfreund and Puerner, 1985). Both MTT and NRU assay results showed that PS-Et induces cytotoxic effects on all the cells in a dose-dependent way. PS-Et exhibited the highest cytotoxicity in A-549 cells (IC_{50} =17.6 $\mu\text{g/mL}$) compared to Caco-2 cells (IC_{50} =21.2 $\mu\text{g/mL}$). These results align with previous studies, signifying the cytotoxic potential of PS-Et against various cancer cell lines *in vitro* (Farshori et al., 2013; Almzaian et al., 2022). Based on

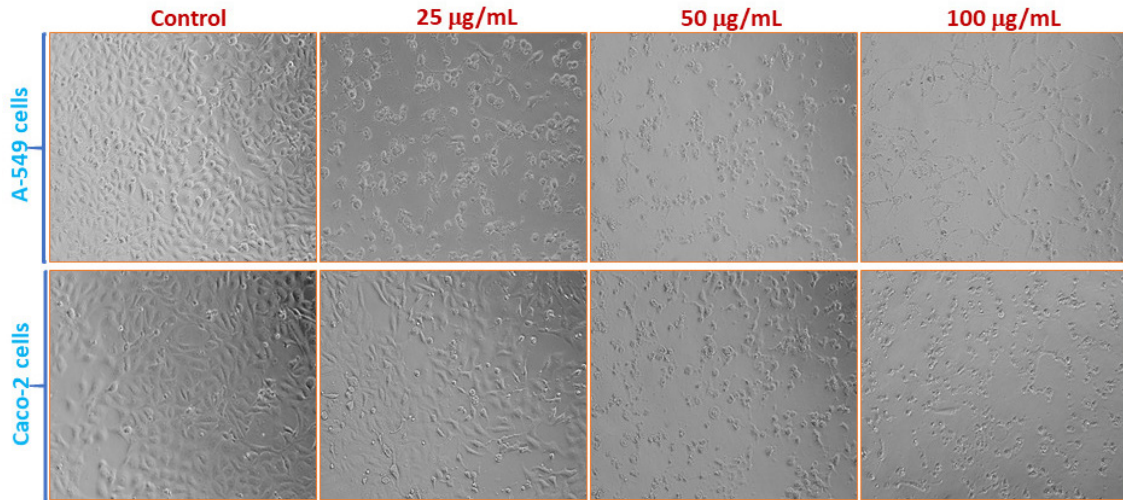


Fig. 3. Morphological visualization of A-549 and Caco-2 cells treated with 25 µg/mL, 50 µg/mL, and 100 µg/mL concentrations of PS-Et, along with the control for 24 h.

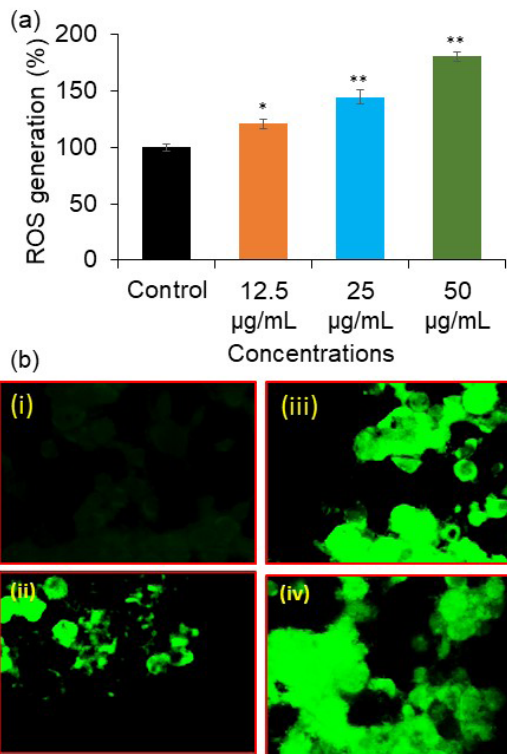


Fig. 4. (a) Photomicrographs illustrating intracellular ROS generation in A-549 cells treated with PS-Et. (b) Graph depicting the level of ROS generation, presented as the percentage of fluorescence intensity relative to the control group. Data are shown as mean ± S.D. * $p < 0.05$ and ** $p < 0.01$, compared to control. (i) control, (ii) 12.5 µg/mL, (iii) 25 µg/mL, and (iv) 50 µg/mL concentrations of PS-Et after 24 h of incubation.

our findings, A-549 cells were chosen for subsequent assays. Further, we assessed cytotoxic effects of PS-Et on cancer cells to evaluate their capacity to induce cell death through morphological analysis. The PS-Et demonstrated anti-proliferative activity and effectively triggered cell death, with the most pronounced effects observed at 24 h of treatment. These findings support a biological mechanism underlying the observed morphological changes, including membrane blebbing, cell shrinkage, cell detachment, and the appearance of apoptotic cells (Srivastava et al., 2015).

Jara-Gutiérrez et al. (2024) reported that oxidative stress is a key mechanism underlying plant extracts-induced cytotoxicity in human cancer cell lines. This is largely attributed to oxidative damage and ROS

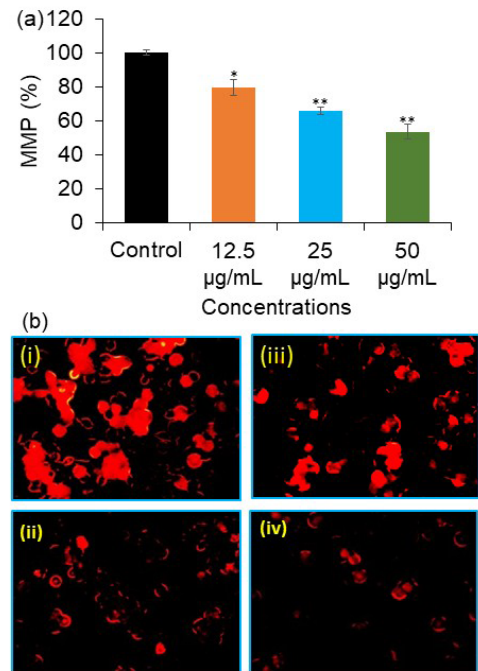


Fig. 5. (a) Fluorescence images showing the MMP level in A-549 cells treated with PS-Et. (b) Graph depicting the MMP level, presented as the percentage of Rh-123 fluorescence intensity relative to the control group. Data are shown as mean ± S.D. * $p < 0.05$ and ** $p < 0.01$, compared to control (i) control, (ii) 12.5 µg/mL, (iii) 25 µg/mL, and (iv) 50 µg/mL concentrations of PS-Et after 24 h of incubation.

production, which are believed to promote the activation of cell death-regulated pathways in cancer cells. In this context, oxidative stress following PS-Et treatment was assessed by quantifying ROS production in A-549 cells. The findings revealed that PS-Et significantly elevated intracellular ROS production, as detected using the cell-permeable dye DCFH-DA. Common oxidative stress includes the generation of free radicals, including superoxide, hydroxyl, and hydrogen peroxide, all of which can damage cellular components, which ultimately leads to apoptotic cell death. In fact, our results indicated that PS-Et induced A-549 cell death could be due to the ROS production. These findings agree with previous studies that reported similar effects in human T leukemia cells (Turrini et al., 2016).

Mitochondria play a vital role in cellular metabolism and energy production, both of which are essential for cell survival (Vakifahmetoglu-Norberg et al., 2017). The mitochondrial membrane potential (MMP) is a critical component in the cascade of events that occur during the

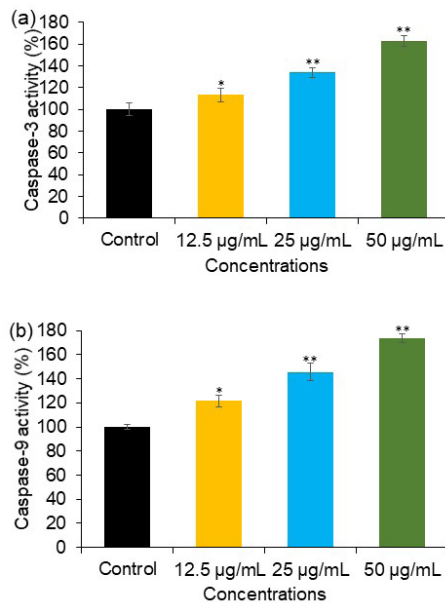


Fig. 6. Effects of PS-Et on (a) caspase-3 and (b) caspase-9 enzyme activities in A-549 cells incubated for 24 h. Data are shown as mean \pm S.D. * $p < 0.05$ and ** $p < 0.01$, compared to control.

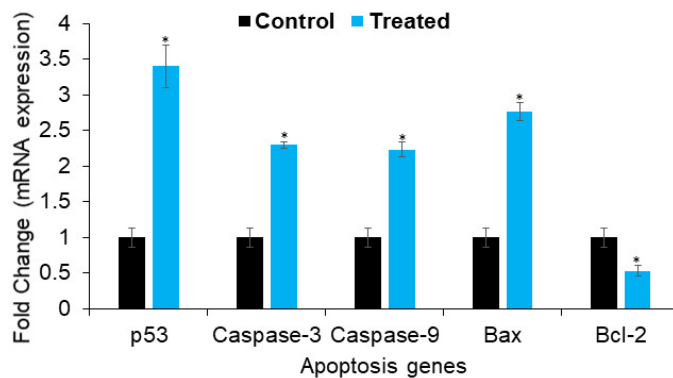


Fig. 7. mRNA expression of apoptotic marker genes (p53, caspase-3, caspase-9, Bax, Bcl-2) in A-549 cells exposed to 25 µg/mL of PS-Et for 24 h. Expression level was observed by qPCR. Data are shown as mean \pm S.D. * $p < 0.05$ compared to control.

intrinsic apoptotic pathway (Zorova et al., 2018). In this study, PS-Et caused a significant disruption of MMP. This disruption specifies that the PS-Et induces apoptosis, as the loss of MMP is a hallmark event in the initiation of mitochondria-mediated apoptosis.

Conversely, caspase-3 and -9 enzyme activities showed a significant concentration-dependent increase of 162% and 173%, respectively, at the highest concentration of PS-Et in A-549 cells. Caspases are a family of cysteine proteases that play a vital role in the apoptotic process (Julien and Wells, 2017). They are expressed in cells as inactive proenzymes and become activated during apoptosis. This activation triggers a cascade that results in the initiation and accomplishment of cell death. As the key executioner caspase, caspase-3 is predominantly activated by initiator caspases, such as caspase-9. Once activated, caspases cleave various cellular proteins, ultimately resulting in apoptosis (Parrish et al., 2013). The present study showed that caspase enzyme activities are associated with apoptosis, evidenced by the increased caspase-3/9 levels corresponding to control in A-549 cells. Caspase activity analysis suggests that apoptosis is likely the main mechanism of cell death caused by PS-Et.

Further examination of the molecular mechanisms underlying PS-Et-induced apoptosis involved the expression of key apoptosis-related genes using real-time PCR. Inducing apoptosis to inhibit cancer cell proliferation is a well-established mechanism of action for many anticancer agents (Alshammari et al., 2020). Apoptosis can be triggered

via either the extrinsic or intrinsic pathway (Ryter et al., 2007). The Bax/Bcl-2 ratio also plays a critical role in regulating the mitochondrial release of cytochrome c and the subsequent activation of caspase-3 (Akbaribazm et al., 2021). Specifically, caspase-3 functions as the key executioner of apoptosis, while caspase-9 acts as an initiator by activating caspase-3 through the mitochondrial-dependent pathway (Jiang et al., 2005). The tumour suppressor gene p53 functions as a transcription factor that regulates both cell growth and apoptosis in response to various forms of cellular stress or damage (Riley et al., 2008). Once release from the mitochondria, cytochrome c interacts with apoptotic protease-activating factor-1 (Apaf-1), forming apoptosome complexes that activate caspases and initiate apoptosis. As shown in Fig. 7, we observed an upregulation in the mRNA levels of pro-apoptotic genes p53, Bax, caspase-3, and caspase-9, and a downregulation of the anti-apoptotic gene Bcl-2 in response to PS-Et treatment, compared to the untreated control. Numerous studies have also demonstrated that plant extracts and phytoconstituent chemicals can activate apoptotic pathways in human cancer cells (Rajabi et al., 2021; Chaudhry et al., 2022). In line with this, our study indicates that PS-Et could be a promising candidate for cancer treatment.

5. Conclusions

Our study revealed that *Petroselinum sativum* ethanolic extract (PS-Et) effectively induced cytotoxic effects in cancer cells. The altered cell morphology is an indication of the strong anticancer potency of PS-Et. Treatment with cytotoxic concentrations of PS-Et led to the generation of ROS. Our results also suggest that PS-Et induces apoptosis in cancer cells through the mitochondrial pathway, which entails a loss of MMP and the activation of caspases-3 and -9. As this study was limited to *in vitro* cancer cell models, further studies involving animal models are needed to better understand the therapeutic and preventive anticancer potential of *Petroselinum sativum*.

CRediT authorship contribution statement

Nida N. Farshori: Conceptualization, methodology, data curation, writing—original draft, review & editing, supervision. **Ebtesam S. Al-Sheddi:** Methodology, data curation, resources, review & editing. **Mai M. Al-Oqail:** Methodology, validation, data curation, review & editing. **Maqsood A. Siddiqui:** Methodology, data curation, writing—original draft.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work presented in this paper.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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