Trop J Nat Prod Res, August 2025; 9(8): 3877 - 3882 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

An *In vitro* Investigation of Cytotoxic Potential of *Platanus orientalis* Leaves on Lung Cancer Cell Line

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ARTICLE INFO

Article history:
Received 13 July 2025
Revised 30 July 2025
Accepted 05 August 2025
Published online 01 September 2025

ABSTRACT

Platanus orientalis is a medicinal plant with numerous biological activities, including wound healing, anti-inflammatory, antioxidant, and anti-proliferative activities. This study aimed to evaluate the anticancer effect of *Platanus orientalis* leaf ethanol extract (PO-EE) against A549 human lung cancer cells and determine the possible mechanism of the extract-induced apoptosis. PO-EE was obtained by Soxhlet extraction in 80% ethanol at 45ºC for 16 hours. Phytochemical constituents of PO-EE were determined using gas chromatography-mass spectrometry (GC-MS). Cytotoxicity of PO-EE was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay, followed by high-content screening for apoptosis detection. Apoptotic mechanism was investigated by evaluating caspases 8 and 9 activities, as well as *BAX* and *Bcl-2* genes expression. GC-MS analysis revealed fatty acids as the major components of PO-EE. Anti-cancer activity screening results showed that PO-EE significantly inhibited the proliferation of A549 cells in a concentration-dependent manner, with an IC_{50} of 65.22 µg/mL. High-content screening confirmed the cytotoxic effects of PO-EE, showing increased nuclear condensation, membrane permeability, loss of mitochondrial membrane potential, and cytochrome *c* release in treated cells. PO-EE induced caspases 8 and 9 activation in A549 cells, indicating the involvement of both extrinsic and intrinsic apoptotic mechanisms. Molecular analysis revealed a 2.85-fold upregulation of the pro-apoptotic *BAX* gene expression and 0.11-fold downregulation of *Bcl-2* gene upon PO-EE treatment. These findings suggest that PO-EE exhibits potent anticancer activity against A549 cells by triggering apoptosis through mitochondrial dysfunction and caspase cascade activation. The findings therefore highlight the therapeutic potential of *P. orientalis* leaf extract, especially for lung cancer treatment.

Keywords: *Platanus orientalis*, A549 cell line, Caspases 8 and 9, MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide), High Content Screening.

Introduction

Medicinal plants have been used as traditional therapies for various ailments since ancient times. The effective pharmacological properties of plant-derived natural products have motivated scientists to investigate and develop promising plant-based treatments against several diseases.[1-4] *Platanus orientalis* L., commonly called Chinar or Oriental plane, is a deciduous, woody, perennial tree in the *Platanaceae* family, and cultivated in southwest Asia.[5] The genus *Platanus* comprise nine species, which are distributed in different parts of the world including Asia, Europe, and America. Different parts of the plant including the seeds, leaves, fruits, and barks, have been used in many countries for both medicinal and non-medicinal purposes. Chinar extracts has been reported to demonstrate anti-inflammatory,[6] anti-aging,[7] and anti-cancer properties.[8] The plant has also been used as a remedy for rheumatic and dermatological disorders, dysentery, diarrhea, asthma, and ocular disorders. [9]

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Citation: Alshanon A, Dawood DAS, Khalaf HY. An *In Vitro* Investigation of Cytotoxic Potential of *Platanus orientalis* Leaves on Lung Cancer Cell Line. Trop J Nat Prod Res. 2025; 9(8): 3877 – 3882 https://doi.org/10.26538/tjnpr/v9i8.49

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Numerous metabolites, including kaempferol-3-*O*-α-l-rhamnopyranoside, kaempferol-3-*O*-*β*-d- glucopyranoside, caffeic acid, proanthocyanidin glycosides, flavonol glycosides, platanoside, tiliroside, fatty acids, and phytol derivatives have been found in different extracts of the plant.[10] Some bioactive flavonoid and triterpenoid compounds with anti-inflammatory and analgesic activities have also been identified from *P. orientalis* extract.[11] In addition, extracts from *P. orientalis* have demonstrated potent antioxidant properties, effectively counteracting various reactive oxygen and nitrogen species.[12] *P. orientalis* extracts have also demonstrated the capacity to mitigate lipid peroxidation, a critical mechanism in the development of illnesses associated with oxidative stress.[13] Furthermore, *P. orientalis* extracts have been shown to enhance the function of endogenous antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase. These enzymes are crucial for protecting the body against oxidative stress.[14] Several *in vitro* studies have revealed the ability of the alcohol and aqueous extracts of *P. orientalis* to reduce the growth of cancer cells, such as MDA-MB-23,[15] and MGC-803.[8] These studies also demonstrated that *P. orientalis* extracts upregulates the expression of apoptotic genes in cancer cells through many pathways, including the production of reactive oxygen species (ROS), activation of caspase enzymes, and inhibition of mitochondrial function.

Lung cancer is one of the common and a predominant form of cancer. Lung cancer was responsible for over 10 million deaths globally in 2018.[16] Resistance to chemotherapeutic agents by cancer cells is the main reason for treatment failure in cancer chemotherapy, resulting in only 18% five-year survival rate.[17] Cancer chemotherapy is also associated with severe side effects, leading to poor patient compliance, and reduced treatment effectiveness. One of the strategies to

overcoming the side effects of chemotherapy is to find alternative medicines that are natural products-based. This study aimed to investigate the chemical composition of *P. orientalis* ethanol leaf extract (PO-EE) for the first time, and to determine its anticancer activity against A549 lung cancer cells, as well as its possible pathway in inducing apoptosis.

Materials and Methods

Chemicals

Ethanol, Eagle's Minimum Essential Medium (EMEM), fetal bovine serum, and dimethyl sulfoxide (DMSO) were products of Sigma-Aldrich (Germany), MTT kit was bought from Abbexa Ltd (UK). Caspase-Glo® 8 and Caspase-Glo® 9 and TRIzol Plus kits were obtained from Promega (USA).

Plant collection and identification

Fresh leaves of *P. orientalis* were collected from the greenhouse of College of Science, Baghdad University, Iraq in October 2023. The plant was identified and authenticated by expert Asst. Prof. Dr. Zainab Abid Aun, Department of Biology, College of Science for Women, Baghdad University, then deposited at Herbarium of College of Science for Women, Baghdad University, where a voucher number 1497 was assigned.

Extraction of plant material

P. orientalis leaves were washed with tap water to remove dirt, and then dried thoroughly. The dried leaves were pulverized into a fine powder using a Wiley Mill grinder (Standard Model No. 3), and subsequently stored in a refrigerator at 4°C.

The powdered sample (100 g) was extracted with 250 mL of 80% ethanol by Soxhlet extraction technique. The extraction was conducted at a temperature of 45°C for 16 hours.[18]

The resulting extract (coded as PO-EE) was concentrated *in vacuo* using a rotary evaporator at 40-45°C until complete evaporation of solvent was achieved. The dried extract was weighed and used to prepare a stock solution, which was subsequently stored in a refrigerator at 4°C.

Phytochemical analysis

The phytochemical constituents of PO-EE were identified using Gas Chromatography – Mass Spectrometry (GC-MS). GC-MS analysis was carried out on Agilent Technologies 7890 Gas Chromatograph (USA) equipped with a capillary column HP-5MS 30 x 0.25 mm inner diameter (Agilent Technologies 19091 S-433) and 70 eV EI mode mass detector (Agilent Technologies model 5975 C). The thermal ramp rates gradually increased the temperature from 60°C to 220°C at a rate of 5°C per minute, and then maintain at 220°C for 10 minutes. The interface temperature was configured to 280°C, while the mass range was set between 30 and 600 m/z. The identification of the compounds was done by comparing the retention times and mass spectra of the compounds with reference compounds in National Institute of Standards and Technology (NIST) database.

Cell Line and cytotoxicity evaluation of PO-EE

Lung adenocarcinoma (A549) and normal HdFn cell lines were obtained from the Biotechnology Research Center – Al-Nahrain University. The cells were cultured in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% fetal bovine serum and 1% penicillin G-streptomycin solution, and maintained at 37°C, 5% CO_2, 95% air, and 100% relative humidity. Cytotoxicity evaluation was conducted on both cell lines using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide (MTT) assay. A volume of 100 µL of the cell suspension was added to each well, containing 1×10^4 cells per well, and incubated for 24 hours. Following incubation, the media containing A549 and HdFn cells were exposed to varying concentrations of PO-EE (25 - 400 µg/mL). Following a 24-hour treatment period, 10 µL of MTT solution was added to each well and the cells were further incubated at 37°C, 5% CO_2 for 4 hours. After incubation, the medium was carefully removed and 100 µL of DMSO was added to each well. Solubilization of MTT into violet formazan was quantified by measuring the absorbance at 570 nm using a microplate reader (BioTek, United States). The experiment was carried out in triplicate, and the results of the MTT assay were expressed as percentage of cell inhibition using the following formula (Equation 1).[19]

$$\text{Cell Inhibition (\%)} = \frac{\text{Abs (Sample)}}{\text{Abs (Control)}} \text{x } 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Detection of apoptosis by high-content screening (HCS)

Morphological changes of A549 cells treated with PO-EE at 100 and 200 µg/mL, including nuclear intensity, cellular membrane permeability, mitochondrial membrane potential and the release of cytochrome *c* were investigated using HCS *in vitro*. The assay was performed according to the manufacturer's protocol - multiparameter cytotoxicity 3 kit (Thermo Scientific™, Pittsburgh, PA, USA). Briefly, after treatment of A549 cells with 100 and 200 µg/mL of PO-EE for 24 hours, the

cells were stained with mitochondria membrane potential (MMP) dye and permeability dye for 30 minutes at 37°C. The cells were subjected to a series of fixation, permeabilization, and blocking before being labelled with a primary antibody against cytochrome *c*. A secondary antibody, DyLight 649 conjugated goat anti-mouse IgG was added to each well and incubated for 60 minutes. The plates were analyzed using the ArrayScan HCS (ThermoScientific, USA).

Caspases 8 and 9 detection

The A549 cells were cultured in 96-well flat plates at a concentration of 1×10^4 cells/well. Cells were incubated at 37°C, 5% CO_2 for 24 hours. After incubation, the cells were washed with pre- warmed PBS and treated with PO-EE at concentrations of 100 and 200 µg/mL. Cells were incubated for an additional 24 hours. Dimethyl sulfoxide (DMSO) was used as the negative control. After the incubation period, the plates were allowed to reach room temperature. Then, 100 µL of Caspase-Glo® 8 and Caspase-Glo® 9 reagents were added separately to each well. The plates were gently shaken for 2 minutes, after which they were placed at room temperature for 30 minutes. The caspases 8 and 9 activities were then assessed by detecting the intensity of light emitted by each sample at a wavelength of 405 nm using an ELISA microplate reader (BioTek, United States).

RNA extraction and quantitative Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR)

After subculturing A549 cells and treating them with PO-EE at a concentration of 200 µg/mL for 24 hours, total cellular RNA was extracted using the TRIzol Plus kit, following the procedure provided by the manufacturer, and described previously.[20] The RNA samples were kept at a temperature of -80ºC until they were used. The process of converting total RNA into complementary DNA (cDNA) was accomplished by usng the RevertAid RT kit and following the manufacturer's instructions. The obtained cDNA was used to analyze the expression of *BAX* gene (Forward:5'GGACGAACTGGACAGTAACATGG'3,Revers:5'GCAAAGTAGAAAAGGGCGACAAC'3), and *Bcl-2* gene (Forward:5'ATCGCCCTGTGGATGACTGAG'3, Revers: 5'CAGCCAGGAGAAATCAAACAGAGG'3), in the ABI StepOne Plus RT-PCR system (ThermoFisher) using the HOT FIREPol EvaGreen qPCR Mix Plus (Solis Bio-Dyne). The expression values were standardized using *β*-actin as a housekeeping gene. The cycle threshold (Ct) approach was employed to determine the mean of the cycle threshold. The variations in fold expression across groups were then computed using the $2^{-\Delta\Delta ct}$ method.

Statistical analysis

Data were expressed as mean ± standard deviation (SD) of triplicate determination. Statistical analysis was performed using GraphPad Prism version 9 (GraphPad Software Inc., La Jolla, CA). Data were subjected to One-Way Analysis of Variance (ANOVA) to determine significant differences among the groups. Significant difference was established at $P \leq 0.05$.

Results and Discussion

Extraction yield and phytochemical constituents of PO-EE

The total yield of ethanol extraction of 100 g of dry weight of *P. orientalis* was 9.45 g corresponding to a percentage yield of 9.45%. The GC-MS analysis identified 26 different compounds representing the major component of PO-EE (Table 1). As shown in Table 1, fatty acids were the main components of PO-EE, with benzene propanoic acid (12.53%) being the dominant fatty acid detected, followed by n-hexadecanoic acid (6.15%), octadecenoic acid (4.3%), m-toluic acid (4.28%), octadecatrienoic acid (3.92%), and others.

Table 1: Phytochemical constituents of *Platanus orientalis* leaves identified by GC-MS analysis

Peak No.	Retention Time (min)	Compound Name	Molecular Formula	Relative Concentration (%)
1	5.917	Carbamic acid	CH_3NO_2	1.19
2	7.432	Phenol,2,6-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	1.02
3	7.813	Adipic acid	$C_6H_{10}O_4$	2.75
4	8.072	2,6-Pyridinediamine	$C_5H_7N_3$	1.54
5	8.427	1,3-Dithiolane, 2-benzyl-2-methyl	$C_{11}H_{14}S_2$	2.86
6	8.713	Quinolinone	C_9H_7NO	1.55
7	8.869	Benzenediol	$C_6H_6O_2$	2.62
8	9.5	5-Hydroxymethylfurfural	$C_6H_6O_3$	2.4
9	10.773	4-Fluoro-2-trifluoromethylbenzoic acid	$C_8H_4F_4O_2$	1.3
10	11.716	N-Phenylmaleimide	$C_{10}H_7NO_2$	1.68
11	11.898	2-Butanone	C_4H_8O	1.69
12	12.339	m-Toluic acid	$C_8H_8O_2$	4.28
13	12.590	Ethanone	C_2H_2O	1.31
14	13.162	Propanamide	C_3H_7NO	2.1
15	13.525	Benzenesulfonic acid	$C_6H_6O_3S$	3.13
16	14.070	Nonane	C_9H_{20}	1.3
17	14.252	2,3-Bornanediol	$C_{10}H_{18}O_2$	1.3
18	15.040	2-Methylisoborneol	$C_{11}H_{20}O$	1.58
19	15.654	5-Chlorovaleric acid	$C_5H_9ClO_2$	1.8
20	16.580	8-Isopropoxy-octanoic acid	$C_{11}H_{22}O_3$	2.8
21	18.121	5-Nitro-2,1,3-benzoxadiazole 3-oxide	$C_6H_3N_3O_4$	1.73
22	19.506	Hexadecanoic acid	$C_{16}H_{32}O_2$	3.42
23	19.575	Benzenepropanoic acid	$C_9H_{10}O_2$	12.53
24	20.129	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	6.15
25	22.016	11-Octadecenoic acid	$C_{18}H_{34}O2$	4.3
26	22.570	9,12,15-Octadecatrienoic acid	$C_{18}H_{30}O_2$	3.92

Phytochemical composition of the ethanol leaf extract of *P. orientalis* has previously been reported, and the plant was shown to exhibit diverse composition of different metabolites mainly a wide array of fatty acids.[21] The presence of these fatty acids possibly enhanced the biological activity and medicinal prospect of *P. orientalis*. These fatty acids may be involved in a wide range of biological actions, including antioxidant, anti-inflammatory, and anti-bacterial effects.[22] On the other hand, the physicochemical characteristics of *P. orientalis* leaves extracts, such as solubility, stability, and bioavailability, are highly influenced by the fatty acid composition.[23]

Cytotoxic activity of PO-EE

The cytotoxic activity of PO-EE was investigated against A549 cells in comparison with normal HdFn cell line using the MTT assay. PO-EE demonstrated a significant ($p < 0.01$) inhibition of the proliferation of A549 cells in a concentration-dependent manner, as compared to HdFn cells (Figure 1). The maximum inhibition of A549 cells was 57.03 ± 4.50% at 400 µg/mL (Figure 1). This effect could be attributed to the diverse chemical composition of the extract. The IC_{50} values of PO-EE for A549 and HdFn cell lines were determined to be 65.22 µg/mL and 338.6 µg/mL, respectively.

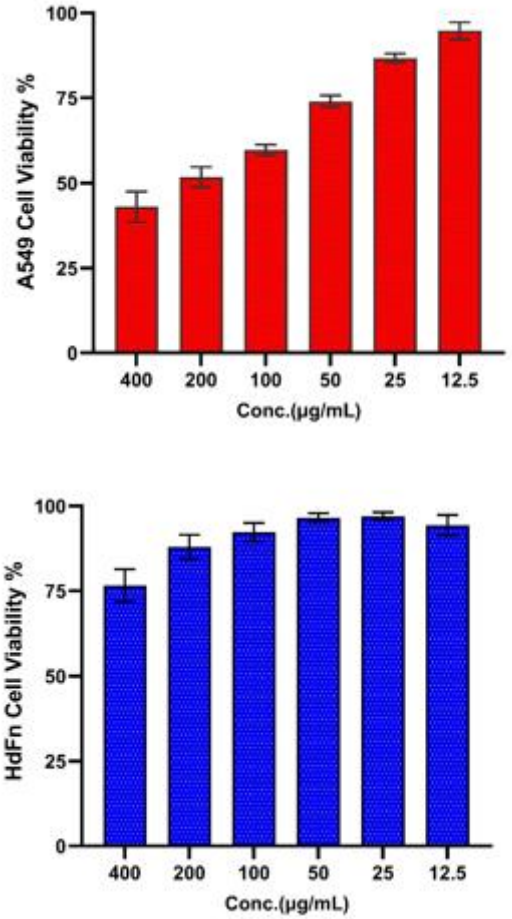

Figure 1: Viability of A549 cells compared with HdFn cells after treatment with different concentrations of ethanol extract of *Platanus orientalis* leaves using MTT assay $n = 3$.

The results from the present study have demonstrated the possible anticancer activity of extracts obtained from *P. orientalis* leaves. More precisely, the ethanol extracts of *P. orientalis* have shown encouraging cytotoxic effects against A549 cancer cells. A study was implemented to assess the cytotoxic effects of both aqueous and alcohol extracts of *P. orientalis* leaves against breast cancer (MDA-MB-231) and normal human fibroblast (L929) cell lines. The results demonstrated that the alcohol extract displayed notable cytotoxic effects against MDA-MB-

231 cancer cells in a concentration-dependent manner, with no comparatively reduced cytotoxicity towards the normal L929 cells.[15] Potent antiproliferative activity of the dichloromethane extract of *P. orientalis* leaves against different cancer cell lines; MDA MB-231, MCF-7, and HeLa cells has been reported.[24]

The cytotoxic effect of *P. orientalis* extract is believed to be due to its abundant phytochemical constituents such as flavonoids, phenolic compounds, and triterpenoids.[25] These bioactive chemicals have been found to have diverse anticancer effects, such as the capacity to trigger apoptosis in cancer cells, hinder cell proliferation, and disrupt cellular signaling pathways that are involved in tumor growth and metastasis.[8]

Apoptotic effect of PO-EE

The results of HCS showed a significant ($p < 0.01$) decrease in total viable count of A549 cells exposed to PO-EE at 100 and 200 µg/mL. The findings strongly indicate that the ethanol extract of *P. orientalis* leaves exhibits cytotoxic effects against A549 cells, as demonstrated by the MTT assay. The decrease in cell count was directly proportional to the concentration of PO-EE, with the most significant ($p < 0.0001$) decrease observed at a concentration of 200 µg/mL (Figure 2A). Figure 2B, C, D and E displays the effects of PO-EE on A549 cell nucleus and mitochondria. These effects were measured using multiple parameters, including nuclear intensity, cell membrane permeability, mitochondrial membrane potential, and cytochrome *c* release. Exposure of A549 cells to increasing concentration of PO-EE led to an increase in nuclear size by 1.3 and 1.5-fold at 100 and 200 µg/mL, respectively, compared to untreated cells (Figure 2B). Furthermore, these events were not triggered by lower concentrations of PO-EE. Significant ($p < 0.01$) increase in cytoplasmic membrane permeability was detected following exposure to only 200 µg/mL of PO-EE with 1.7-fold increase, compared to control (Figure 2C). Additionally, two other metrics, mitochondrial membrane potential and cytochrome *c* release, were also quantified. The results showed that at 100 and 200 µg/mL PO-EE caused a significant ($p < 0.01$) decrease in mitochondrial membrane potential, with percentage decrease of 11.60 and 24.11%, respectively (Figure 2D). Finally, the release of cytochrome *c* was significantly elevated ($p = 0.0457$ and 0.0008) when A549 cells were exposed to 100 and 200 µg/mL of PO-EE (Figure 2E).

Cell-based high-content screening is regarded as a more accurate assay in comparison to traditional MTT cytotoxicity. This method encompasses a wide array of influences and tracks various harmful consequences within a single cell. It quantitatively measures numerous characteristics associated with toxicity.[26] Thus, this assay was utilized to observe the various parametric impacts of PO-EE on A549 cells. The decrease in viable A549 cells at increasing concentrations of the extract may be due to the stimulation of cellular death. The concentrated and vibrant intensity of the detected stains was linked to morphological features associated with cellular death. The subsequent observations were characteristic traits of the morphology of apoptotic cells. The process of apoptosis involves nuclear condensation, nuclear fragmentation, cell shrinkage, and the creation and aggregation of apoptotic bodies.[27] Furthermore, the use of a dye that measures membrane permeability revealed a significant increase in intensity, particularly at the highest concentration of exposure. This finding provides evidence that the extract could trigger apoptosis in A549 cells.

Effect of PO-EE on Caspases 8 and 9 activities

The induction of caspases 9 and 8 in A549 cells treated with 100 and 200 µg/mL PO-EE was estimated. Results showed that PO-EE increased the activity of both caspases 9 and 8 in concentration-dependent manner (Figure 3). Compared to untreated cells, PO-EE treated cells showed 2.24-fold ($p = 0.0311$) and 7.40-fold ($p = 0.0003$) increase in caspase 9 activity at 100 and 200 µg/mL, respectively. While the activity of caspase 8 showed 4.10-fold ($p = 0.0234$) and 8.40-fold ($p = 0.0018$) increase in PO-EE treated cells at 100 and 200 µg/mL, respectively. The study examined the molecular process by which PO-EE triggers apoptosis by activating effector caspases 8 and 9. PO-EE treatment of A549 cells effectively enhanced the activation of caspases 8 and 9, resulting in the stimulation of both intrinsic and extrinsic mechanisms of cell death. This effect was observed in a concentration-dependent manner.

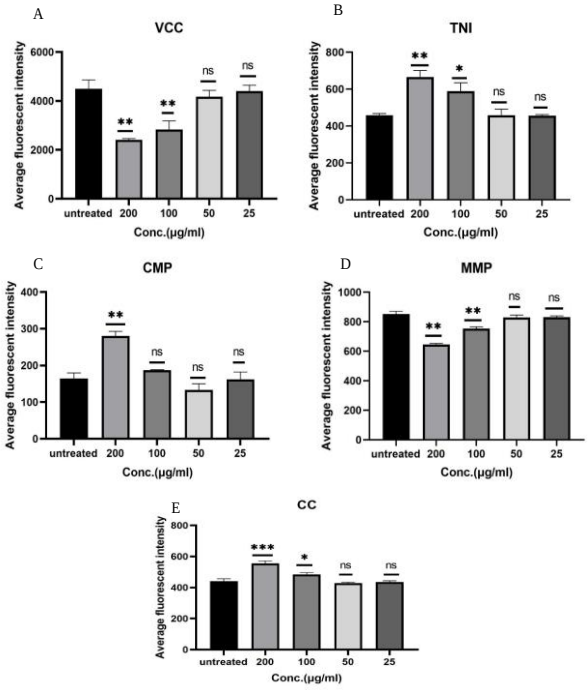

Figure 2: Effects of ethanol extract of *Platanus orientalis* leaves on A549 cell line after treatment for 24 h on; Viable Cell Count, Total Nuclear Intensity, Cytoplasmic Membrane Permeability, Mitochondrial Membrane Potential and Cytochrome *c* Release. The effect was evaluated on the ArrayScan HCS reader. Data represent mean ± SD of three replicate experiment (n = 3), significance differences were observed between the mean values of treated cells and the untreated cells. *: $p < 0.05$, **: $p < 0.01$, NS: Non-Significant.

The elevated level of caspase 8 strongly indicates that treatment with PO-EE triggers the activation of caspase 8. Moreover, the liberation of cytochrome *c* into the cytosol is associated with the initiation of the intrinsic cell death pathway through the activation of caspase 9, which may result in the activation of caspase 7.[28] Given that caspase 9 was induced by PO-EE, it is highly probable that the treatment of A549 cells with PO-EE ultimately triggered the mitochondrial cell death pathway. Previous research indicated that flavanols isolated from the leaves of *Platanus* methanol extract stimulated the generation of reactive oxygen species (ROS), reduced the mitochondrial membrane potential, and triggered the activation of caspase-9 and caspase-3, resulting in cellular apoptosis in different cancer cell lines.[8] Also, compounds isolated from *P. orientalis* exhibited apoptotic induction in human leukemia HL-60 cells by activation of caspases 9 and 8.[29]

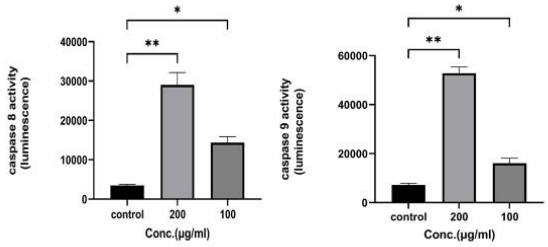

Figure 3: Caspases 8 and 9 expression in A549 cells treated with 100 and 200 µg/mL of ethanol extract of *Platanus orientalis* leaves compared with untreated cells (Control). *: $p < 0.05$, **: $p < 0.01$.

Effect of PO-EE on the expression of BAX and Bcl-2 genes

The expression of *BAX* gene in A549 cells treated with 200 µg/mL PO-EE for 24 hours was detected using qRT-PCR. Compared to untreated cells, treated A549 cells showed significant ($p < 0.0001$) upregulation in *BAX* gene expression of 2.85 ± 1.32-fold increase compared with untreated cells. Conversely, a downregulation of *Bcl-2* gene expression was detected in A549 cells treated with 200 µg/mL PO-EE (0.11 ± 0.03-fold decrease) (Figure 4). The molecular investigation of the effect of PO-EE on gene expression correlated with apoptosis exhibited by the extract, with a high expression of *BAX* and downregulation of *Bcl-2*. *BAX* is an apoptotic gene that correlates with cell death (apoptosis), which implies that the ability of PO-EE to inhibit the proliferation of A549 cancer cells could be attributed to the upregulation of *BAX* gene. The process of apoptosis is a highly regulated process that controls and maintains the cell population in a balanced state by excluding the damaged or abnormal cells, including cancer cells.[30] In contrast, *Bcl-2*, a family of genes that act as anti-apoptotic genes that initiate cell proliferation,[31] and PO-EE effectively inactivated the *Bcl-2* gene in A549 cancer cells. The upregulation of *BAX* as a result of PO-EE exposure suggests that the extract activates both the intrinsic and extrinsic cellular apoptotic pathways in A549 cancer cells (confirmed by caspases 9 and 8 activities), leading to activation of vital regulators of apoptosis, mitochondrial membrane destruction and the release of ROS and cytochrome *c* and activation of caspases.[32]

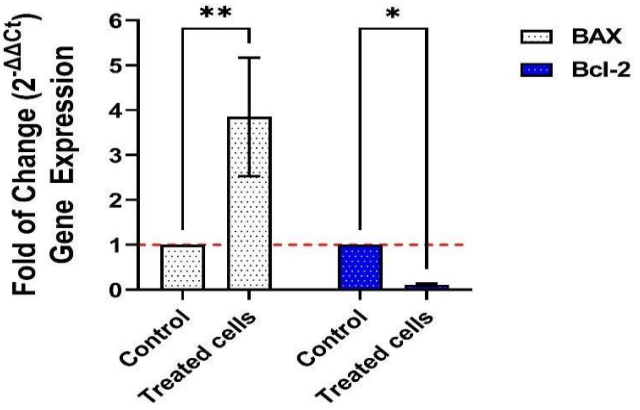

Figure 4: Expression of *BAX* and *Bcl-2* genes in A549 cells treated with 200 µg/mL of ethanol extract of *Platanus orientalis* leaves compared with untreated cells (Control). *: $p < 0.05$, **: $p < 0.01$.

Conclusion

Findings from the present study showed that the ethanol extract of *P. orientalis* (PO-EE) exhibits potent cytotoxic effects against A549 lung cancer cells by inducing apoptosis through the upregulation of *BAX* gene expression, activation of caspases 8 and 9, and disruption of mitochondrial membrane potential. These findings highlight the promising anticancer potential of *P. orientalis* against various cancer cell lines and other pharmacological applications.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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