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# The effect of purified condensed tannins of the flowers of plane trees on the malignant activities of breast cancer cells

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## Abstract

### Objective

Platanus (plane tree) flowers are rich in condensed tannins (CTs), which have been reported to possess various bioactive properties. However, their specific anti-cancer effects on triple-negative breast cancer cells remain poorly characterized. This study aimed to evaluate the dose-dependent effects of purified Platanus flower CTs on cellular proliferation, migration, and the expression of key malignant-related genes (PCNA and MMP9) in MDA-MB-231 breast cancer cells.

### Materials and methods

MDA-MB-231 cells were treated with serial concentrations of purified Platanus flower CTs (1.25, 2.5, and 5 mg/mL). Cellular proliferation was assessed by calculating doubling time using time-lapse microscopy and ImageJ single-cell tracking. Migration was evaluated using a wound-healing assay. Gene expression levels of Proliferating Cell Nuclear Antigen (PCNA) and Matrix Metallopeptidase 9 (MMP9) were measured by RT-qPCR. Cell division rates and wound-healing

migration were continuously monitored using the Axio Imager time-lapse system. Statistical analyses were conducted using GraphPad Prism software.

**Results**

Time-lapse analyses showed a clear dose-dependent response. Higher CT concentrations (2.5 and 5 mg/mL) induced complete cell death within the first hour. Treatment at 1.25 mg/mL produced a potent cytostatic effect without acute toxicity, significantly extending the doubling time from approximately 18 hours (control) to approximately 22 hours ($P < 0.05$). The wound-healing assay revealed significantly reduced wound closure (65-75%) after 24 hours compared to control (80-95%; $P < 0.05$). Molecular analysis demonstrated that 1.25 mg/mL CTs downregulated PCNA expression by 65% and MMP9 expression by 40%. The pattern of differential gene expression observed herein is fully concordant with both proliferation and migration assays.

**Conclusions**

Purified condensed tannins isolated from Platanus flowers demonstrate significant anti-malignant activity against MDA-MB-231 triple-negative breast cancer cells through a dual mechanism: suppressing proliferation via PCNA downregulation and inhibiting migration via MMP9 downregulation. These findings support the potential of Platanus flower CTs as promising candidates for anti-cancer drug development.

**Keywords:** CTs, MDA, PCNA, plane trees, time-lapse

**Paper Type:** Research Paper.

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## Introduction

Phytobiotics and medicinal plants have gained considerable attention in recent years due to their potential as natural alternatives to synthetic additives in nutrition. These natural products are rich in bioactive compounds such as essential oils, alkaloids, flavonoids, and phenolic acids, which contribute to their antimicrobial, antioxidant, and anti-inflammatory properties

(Amirteymoori et al., 2021). Consequently, phytobiotics play a crucial role in improving health, performance, and life quality. The use of phytobiotics and medicinal plants as natural antimicrobial growth promoters in place of antibiotics in feed offers numerous advantages. These benefits include improved zootechnical efficiency parameters, suppression of specific diseases, antimicrobial anticancer, and antioxidant activities, hypocholesterolemic effects, enhancement of digestive enzymes, and improved liver function. Moreover, phytobiotics have been shown to modulate gut microbiota, which enhances nutrient absorption and supports overall immune function. Studies have demonstrated that incorporating these plants into the diets can increase performance and prevent diseases and cancer (Alhasoon et al., 2026). Furthermore, phytobiotics are associated with reducing stress-related impacts. Given the growing global concerns regarding antibiotic resistance and the demand for safer and healthier products, the incorporation of phytobiotics and medicinal plants into feed presents a promising and sustainable alternative strategy (Mohammadabadi et al., 2022). Platanus Orientalis, A species of plane tree (Platanus spp.), is native to regions across Eurasia, extending from southeast Europe to southwest Asia (Sabr, 2021; Amedi et al., 2022). This species has a history of applications in traditional medicine and the pharmaceutical field. The leaves, bark, and flowers of Platanus contain a range of bioactive compounds, notably flavonoids and condensed tannins, which can be extracted using various solvents and have been studied for their anti-inflammatory, antimicrobial, and wound-healing properties (Das et al., 2020; Danika et al., 2024; Li et al., 2023). Chemical analyses of Platanus extracts indicate that the concentration of condensed tannins (CTs) varies considerably depending on the extraction method, solvent used, and the specific plant part harvested (Khan et al., 2013; Shay et al., 2017). Previous studies have reported anti-tumour and cytotoxic activity of CTs against various cancer cell lines, including dose-dependent reductions in the viability of the MDA-MB-231 breast cancer cell line (Florento et al., 2012; Al-Bedhawi et al., 2024). Tannins represent a broad group of polyphenols found in numerous plant species (Crozier et al., 2006). Condensed tannins, also referred to as non-hydrolysable tannins or proanthocyanidins, constitute a specific subgroup within this family (Schofield et al., 2001). Early investigations suggested that tannins exerted adverse effects on animal growth rates, feed intake, and feed efficiency, and were associated with reduced protein digestibility in experimental models (Barry & McNabb, 1999; Larrain et al., 2007). Although one early study proposed a carcinogenic potential for tannins, subsequent research clarified that such effects were attributable to other associated compounds rather than to tannins themselves (Chung et al., 1998). More recent studies have provided substantial evidence that polyphenols possess considerable anti-cancer properties. Apple source polyphenols were suggested to induce a reduction in the expression of UHRF1 and matrix metalloproteinases (MMPs). These two proteins are well established in cancer invasion (Ahmed et al., 2025; Jain et al., 2025; Wu et al., 2018). Zhao et al. (2018) demonstrated that urolithin, which is a major polyphenolic metabolite, activated the autophagy pathway and inhibited metastasis in human colorectal cancer cells (Zhao et al., 2018). Karakurt and Adali (2016)

examined the effects of tannic acid on the proliferative, metastatic, and invasive characteristics of prostate cancer cells, reporting significant dose-dependent inhibition of cell migration, proliferation, and invasion (Karakurt & Adali, 2016). The botanical origin of tannins may also contribute to variation in their anti-cancer activity. For example, tannins purified from Spatholobi Caulis exhibited anticancer properties by inhibiting the protein synthesis required for the transition of HeLa cells from the G0/G1 to the S phase of the cell cycle (Wang et al., 2018; Smeriglio et al., 2017). Building on this body of evidence, the present study aimed to examine the effects of highly purified CT extracts derived specifically from Platanus flowers on key cellular and molecular parameters of breast cancer cells.

## Materials and methods

**Extraction and Purification of Condensed Tannins (CTs):** Platanus flowers were used as the primary material for CTs, after collection, characterization, and identification in the chemical laboratory. Tannins were extracted from the Platanus flowers using an acetone/water solvent mixture (1:1, v/v) (Williams et al., 2014). Then crude extracts were purified using gel filtration chromatography using a column of Sephadex LH-20 (Cytiva,Marlborough, USA). The extracted solution was then loaded in the column, followed by a washing step with an acetone/water mixture (8:2, v/v) to elute the highly purified non-hydrolysable tannins (Brown et al., 2017). Purified samples were quantified by thiolysis degradation. Molecular compositions and CT contents were confirmed by high-performance liquid chromatography-mass spectrometry (HPLC/MS). All experimental procedures were carried out in accordance with standard regulations and guidelines for biological assays. The ethical framework for in vitro cell line testing was followed, as established by the ethics committee at the Institute of Genetic Engineering and Biotechnology, University of Baghdad, Baghdad, Iraq.

**Cell Line Preparation and CT Serial Concentrations:** The MDA-MB-231 breast cancer cell line was used as a model to assess the efficacy of the extracted CTs. Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Waltham, USA) was used for cell culture, which was supplemented with penicillin (100 U/mL), streptomycin (100 µg/mL), L-glutamine (2 µM), and 10% (v/v) foetal calf serum. Trypan blue exclusion staining was used to assess cell viability. Cultures were maintained at 37°C in a humidified atmosphere with 5% $CO_2$. The migration assay started when cells reached 70-90% confluence. The cells were trypsinized with TrypLETM Express (Gibco, Waltham, USA) and seeded into 6-well plates. At the same time, the proliferation assay started when the cell culture reached 30-50% confluency before CT treatment, and the time-lapse assay. For cells designated for migration assay, as they reach 80-90% confluency, a scratch wound was made using sterile 100 µl pipette tips. Treatment with purified Platanus CTs was conducted using serial concentrations of (1.25 mg/mL, 2.5 mg/mL, and 5 mg/mL) diluted in DMEM immediately before application. Cell proliferation was assessed by calculating doubling time. Both cell division (proliferation) and migration (via a wound healing assay) were monitored

using an automated Axio Imager time-lapse system programmed to capture images every 10 minutes over a period exceeding 24 hours. The analysis of proliferation was carried out using ImageJ software for single-cell tracking to calculate doubling time. The analysis of migration was also carried out using ImageJ software to measure residual wound area at defined time points for calculating the percentages of wound closure at each of these time points.

**RNA Extraction and cDNA Synthesis:** To examine the molecular effects of the Platanus CT extracts, total RNA was extracted from treated and control MDA-MB-231 cells using a Total RNA Extraction Kit (Addbio, Daejeon, Korea), following the manufacturer's protocol. RNA concentration and purity were measured before downstream use. Complementary DNA (cDNA) was then synthesized from the total RNA using a cDNA Synthesis Kit (Addbio, Daejeon, Korea) to provide a stable template for real-time PCR.

**Real-Time PCR Analysis**: Real-time quantitative PCR (RT-qPCR) was performed to measure expression levels of target genes associated with cell proliferation and migration. Proliferation was assessed by quantifying transcript levels of Proliferating Cell Nuclear Antigen (PCNA; NM_002592) using the following primers: Forward: GCCCTGGTTCTGGAGGTAAC Reverse: TAGCTGGTTTCGGCTTCAGG. Migration was assessed at the molecular level by measuring matrix metallopeptidase 9 (MMP9; NM_004994) expression using: Forward: TCTATGGTCCTCGCCCTGAA Reverse: CATCGTCCACCGGACTCAAA. Beta Actin (Forward: TCACCATGGATGATGATATCGC, Reverse: ATAGGAATCCTTCTGACCCATGC) was used as the endogenous reference gene for normalization. All primers were purchased from ALPH Canada, Toronto, Canada. Relative fold changes in gene expression were calculated using the ΔΔCt method.

**Data Acquisition and Statistical Analysis**: Cell division rates and wound-healing migration were continuously monitored using the Axio Imager time-lapse system, which acquired images at 10-minute intervals over 24 hours. ImageJ was used for single-cell tracking and wound area measurements at defined time points to calculate wound healing percentages.

**Statistical analysis**: All experiments were performed in triplicate (in = 3 independent biological replicates). Data are presented as mean ± standard deviation (SD). Statistical analyses were conducted using GraphPad Prism software (version 9.0, GraphPad Software, San Diego, CA, USA). Comparisons between two groups were analyzed using Student's t-test. Comparisons among multiple groups were assessed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. Statistical significance was defined as $P < 0.05$.

## Results

**Analysis of Proliferation Dynamics**: Proliferation dynamics are summarized in Table 1. In the untreated control, MDA-MB-231 cells proliferated continuously throughout the observation period. Time-lapse imaging captured the typical morphological changes of the cell cycle: cells regularly rounded up during mitosis, divided into two daughter cells, and subsequently

flattened and re-adhered to the plate. Throughout the visualization, estimates of the entire field of view, cell divisions took place repeatedly, leading to a notable rise in confluence from the 0- to 24-hour time points. When cells were exposed to the highest CT concentrations (5 mg/mL and 2.5 mg/mL), this induced a fatal effect. No surviving cells were observed after the first hour of culture (Figure 1). The result changed considerably at 1.25 mg/mL. Cells that survived after treatment remained attached to the surface, maintained their elongated shape, and continued to send out pseudopodia while actively migrating. The rate of cell division was significantly lower than that in the untreated control. A minority of mitotic events were observed, but the majority of cells appeared to be held in an interphase-like state for the duration of the observation period. Cell tracking analysis using ImageJ confirmed that 1.25 mg/mL CTs significantly ($P<0.05$) decreased cancer cell doubling time relative to the untreated control. The higher concentrations (2.5 and 5 mg/mL) were cytotoxic, with cell death occurring within hours of treatment. This anti-proliferative effect is consistent with known mechanisms by which tannins bind proteins and disrupt key cancer cell signaling pathways. Accordingly, the increase in cell density over the 24-hour period was considerably lower in the 1.25 mg/mL treatment group than in the control. Taken together, imaging data and quantitative cell tracking support the conclusion that 1.25 mg/mL Platanus CTs exert a cytostatic effect on MDA-MB-231 cells without inducing acute cytotoxicity.

**Table 1. Analysis of the observational, morphological, and proliferation results**

| Observation and Image J Metric | Control Group | 1.25 mg/mL CTs |
|---|---|---|
| Mitotic Frequency | High; frequent visual evidence of cells rounding up and dividing. | Low; mitotic events are rare and sporadic. |
| Change in Cell Density | Significant increase in confluence from 0 h to 24 h. | Minimal increase in confluence; cell numbers remain relatively stable. |
| Cell Morphology | Healthy, typical adherent spindle/polygonal shapes. | Viable and adherent; no signs of membrane blebbing or structural collapse. |
| Cell Viability | High; no obvious cell death or floating debris. | High; cells remain alive and motile, indicating no acute direct toxicity. |
| Overall Proliferation Status | Normal, rapid exponential growth. | Highly suppressed (cytostatic effect). |
| Doubling time (image J) | 18 hours: 40 minutes ± 60 min | 21 hours: 45 minutes ± 30 min |

Note: Data are presented as mean ± standard deviation (SD) from three independent experiments (n=3). Doubling time was calculated using single-cell tracking in ImageJ. *$P < 0.05$ compared to the control group (Student's t-test).

**Analysis of Migration Dynamics**: The migration dynamics results are presented in Table 2. At zero (0 hours), the control group cells were clearly visible on either side of the initial scratch wound, with a well-defined, cell-free gap running through the center of the field of view. Over the course of the time lapse, cells at the leading edges actively migrated into the denuded area, undergoing division as they advanced. By 24 hours, the wound was almost entirely closed, with cells having repopulated the gap to form a dense, confluent monolayer, leaving virtually no empty space. In contrast, cultures treated with the higher concentrations of purified tannins from Plane tree flowers (5 mg/mL and 2.5 mg/mL) exhibited a pronounced cytotoxic effect, with complete cell death occurring within the first hour of culture (Figure 2). At the lowest concentration tested (1.25 mg/mL), the scratch appearance at 0 hours was comparable to that of the control, with a clear, cell-free zone visible across the field. Despite maintaining viability and motility throughout the experimental period, with cells demonstrating extension into the cleared region, the coordinated directional displacement toward the wound center was substantially diminished in treated cultures relative to untreated controls. At the 24-hour terminal observation point, data from the wound-healing assay were consistent with proliferation outcomes, indicating that elevated concentrations of the condensed tannin (CT) exerted a pronounced inhibitory effect on both migratory behavior and wound-closure efficiency compared with the control and sub-threshold concentration groups. Quantitative evaluation via ImageJ software indicated an absence of statistically significant divergence in wound closure ratio at the minimal CT concentration following the 12-hour interval, wherein closure ranged between 21-32%, in contrast to the 26-37% documented among control specimens. Nevertheless, at the 24-hour endpoint, a statistically significant attenuation ($p < 0.05$) in wound closure was observed at the lowest treatment concentration (65-75%) in comparison to the control cohort (80-95%). Collectively, the present findings demonstrate that the investigated CT compounds harbor both anti-proliferative and anti-migratory capacities, thereby underscoring their prospective utility as viable candidates warranting further systematic evaluation within the domain of anticancer pharmaceutical development. The migration results are consistent with the proliferation data. In vitro wound healing depends on two cellular processes: motility (movement of cells into the cleared space) and proliferation (cell division to repopulate the area). At 1.25 mg/mL, the tannin concentration produced a marked cytostatic effect, substantially reducing cell division.

**Gene expression of target genes**: To better understand the molecular basis of the anti-proliferative and anti-migratory effects observed with Platanus flower CTs, the expression of key genes involved in these processes was investigated. The transcript levels of Proliferating Cell Nuclear Antigen (PCNA), a well-established marker of cell proliferation, and Matrix Metallopeptidase 9 (MMP9), an enzyme with a central role in cell migration and invasion, were measured. The analysis was carried out at the non-cytotoxic concentration of 1.25 mg/mL CTs, as the higher concentrations tested (2.5 and 5.0 mg/mL) caused rapid cell death, making gene expression analysis unfeasible.

**Table 2. Qualitative and quantitative analysis of the migration assay**

| Observation Metric | Control Group | 1.25 mg/mL CTs |
|---|---|---|
| Edge Activity | Highly active; rapid extension into the gap. | Slower, less aggressive extension into the gap. |
| Wound Closure Rate | Fast. | Visibly delayed/inhibited. |
| Final Wound Status | Nearly completely closed (high %). | Significant $p < 0.05$ gap remains open (lower %). |
| Cell Density in Wound | High; gap is filled with closely packed cells. | Low; cells in the gap are sparse. |
| Wound healing (image J) | 26-32% after 12 hours | 21-32% after 12 hours N. S |
| | 80-95% after 24 hours | 65-75% after 24 hours, $p < 0.05$ |

Note: Wound closure percentages are presented as ranges from three independent experiments (n=3). N.S. = not statistically significant ($P > 0.05$). *$P < 0.05$ compared to the control group at 24 hours (one-way ANOVA followed by Tukey's post-hoc test).

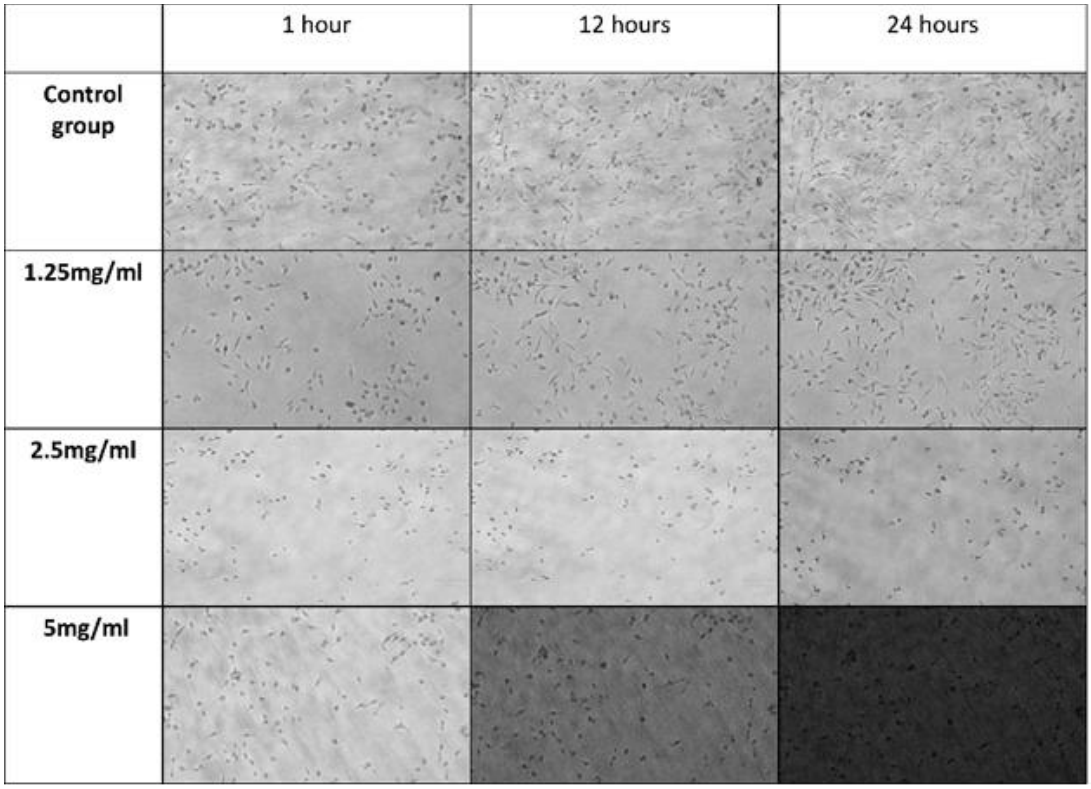


**Figure 1. Effect of CTs purified from flowers of the Plane tree on MDA cell proliferation. MDA cells were cultured to 30-50% confluence prior to exposure to serial concentrations of CTs (untreated control, 1.25 mg/mL, 2.5 mg/mL, and 5.0 mg/mL). Cellular proliferation was continuously monitored utilizing a cell tracking assay with time-lapse microscopy (10x magnification) acquired at 10-minute intervals. The analysis revealed a baseline doubling time of approximately 18 hours for the untreated control cells. Administration of 1.25 mg/mL CTs significantly extended the doubling time to around 22 hours. Conversely, exposure to higher concentrations (2.5 mg/mL and 5.0 mg/mL) completely arrested cellular proliferation and induced cell death within the initial hours of treatment.**

The effect of the CT extract on PCNA expression is shown in Table 3. Compared to the untreated control, PCNA mRNA levels in treated cells were reduced by 65%. This downregulation of PCNA is in line with the extended doubling time and cytostatic effect recorded in the proliferation assays, indicating that CTs suppress cancer cell growth, at least partly, through inhibition of this key proliferation-associated gene. The modulatory effect of CT treatment upon the transcriptional activity of the migration-associated gene MMP9 is presented in Table 4. Quantitative assessment of fold change in MMP9 transcript abundance yielded a value of 0.6, corresponding to a 40% reduction relative to the untreated control group. This transcriptional suppression of MMP9 furnishes a plausible mechanistic rationale for the attenuated migratory behavior documented in the wound-healing assay. The impaired capacity of cells to reconstitute the scratch wound may be ascribed, at least in part, to the substantially reduced expression of this serine-independent metalloprotease, whose enzymatic function in extracellular matrix proteolysis is indispensable for facilitating directional cell motility.

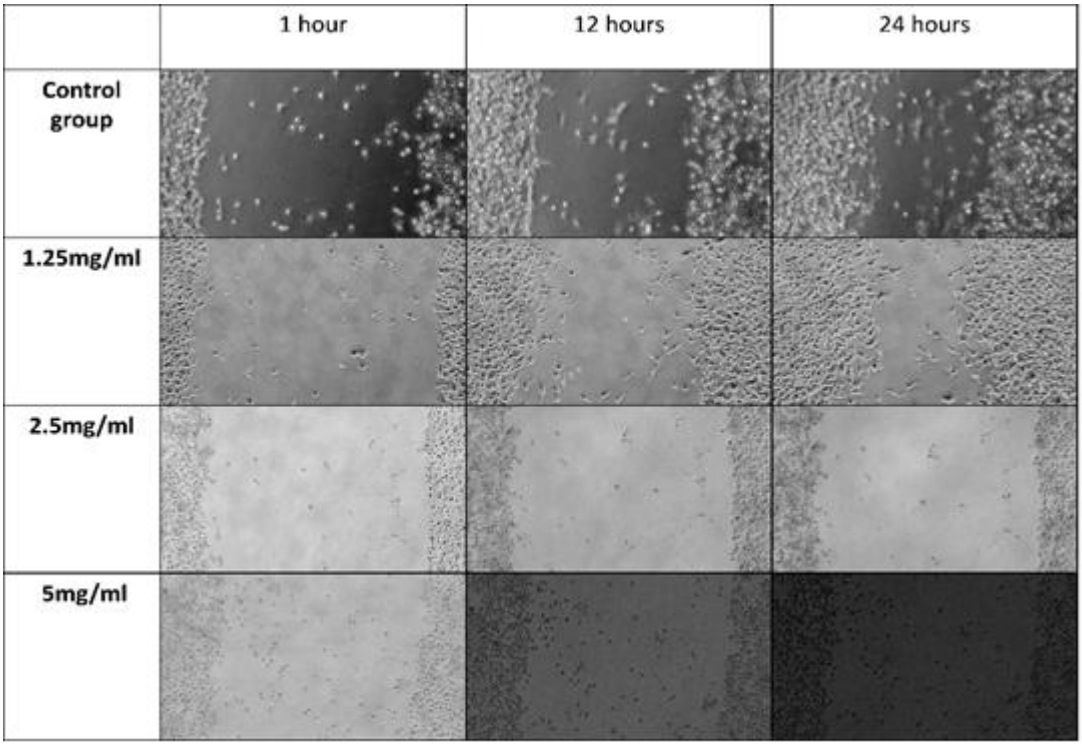


**Figure 2. Dose-dependent inhibition of MDA cell migration by CTs purified from flowers of the plane tree in a wound-healing assay. Cell monolayers were cultured to complete confluence prior to mechanical scratch wounding. Following exposure to serial concentrations of CTs (untreated control, 1.25 mg/mL, 2.5 mg/mL, and 5.0 mg/mL), migration was continuously monitored via time-lapse microscopy (10x magnification) with image acquisition at 10-minute intervals. CT treatment significantly attenuated the migratory capacity of the cells compared to the untreated control in a strictly dose-dependent manner**

**Table 3. Fold change in gene expression of PCNA normalized to β-actin gene in MDA cells calculated by the Livak method**

| Group | Mean Δ Ct (PCNA Ct - *β-actin* Ct) | Mean ΔΔ Ct | Mean $2^{-\Delta\Delta Ct}$ | Fold difference in gene expression |
|---|---|---|---|---|
| Control cells | 3 | 0 | 1 | 1 |
| Cells treated with 1.25mg/mL CTs | 4.51 | 1.51 | 0.35 | 0.35 |

Note: Gene expression was normalized to β-actin as the endogenous control. Values represent means from three independent experiments (n=3). Fold change was calculated using the $2^{-\Delta\Delta Ct}$ method.

Collectively, the molecular analyses demonstrated that exposure to a sub-cytotoxic concentration of purified condensed tannins derived from Platanus floral tissue (1.25 mg/mL) induced pronounced transcriptional suppression of both PCNA (65% reduction) and MMP9 (40% reduction). The pattern of differential gene expression observed herein is fully concordant with both proliferation and migration assays. These findings offer a substantive mechanistic underpinning for the phenotypic inhibition of proliferative and migratory activities documented in the breast cancer cell model.

**Table 4. Fold change in gene expression of MMP9 normalized to the β-actin gene in MDA cells calculated by the Livak method**

| Group | Mean Δ Ct (MM9 Ct - *β-actin* Ct) | Mean ΔΔ Ct | Mean $2^{-\Delta\Delta Ct}$ | Fold difference in gene expression |
|---|---|---|---|---|
| Control cells | 7 | 0 | 1 | 1 |
| Cells treated with 1.25mg/mL CTs | 7.74 | 0.74 | 0.6 | 0.6 |

Note: Gene expression was normalized to β-actin as the endogenous control. Values represent means from three independent experiments (n=3). Fold change was calculated using the $2^{-\Delta\Delta Ct}$ method.

**Discussion**

The present investigation evaluated the antiproliferative and antimigratory potential of purified condensed tannins (CTs) obtained from Platanus spp. floral tissue, employing the MDA-MB-231 triple-negative breast cancer cell line as an experimental model (Huang et al., 2020). Collectively, the findings demonstrate that the aforementioned CTs exert concentration-dependent inhibitory effects on both cellular proliferation and migratory capacity. Of considerable significance, the observed phenotypic alterations are substantiated by quantifiable molecular

evidence: at a sub-cytotoxic concentration, concurrent downregulation of proliferating cell nuclear antigen (PCNA) and matrix metalloproteinase-9 (MMP9) (Romero Romero et al., 2022; Wang et al., 2025). Quantitative proliferation analyses revealed a well-defined dose-dependent inhibitory response across the concentration range examined. The two highest concentrations of 2.5 mg/mL and 5.0 mg/mL induced rapid cytotoxic outcomes. While treatment with 1.25 mg/mL elicited a predominantly cytostatic response, manifested as a prolongation of the cell doubling interval from approximately 18 hours to nearly 22 hours ($P<0.05$). Corroborating these phenotypic observations at the molecular level, quantitative gene expression analysis demonstrated a 65% attenuation of PCNA transcript abundance in extract-treated cells relative to untreated controls. Given that PCNA functions as an indispensable processivity cofactor for DNA polymerase delta during chromosomal replication and constitutes a well-validated proliferative biomarker, its transcriptional attenuation furnishes a mechanistically coherent explanation for the retarded growth kinetics recorded in the proliferation assays (Kang et al., 2024; Kelman, 1997; Stoimenov & Helleday, 2009). This observation is further contextualized within the broader literature on polyphenolic bioactivity. Wu et al. (2018) demonstrated that apple-derived polyphenols attenuated the malignant phenotype of MDA-MB-231 cells, an effect mechanistically attributed to diminished expression of UHRF1, a regulator functionally intertwined with PCNA-mediated cell cycle progression (Wu et al., 2018). Analogously, tannic acid has been documented to suppress prostatic carcinoma proliferation in a concentration-dependent manner (Karakurt & Adali, 2016). The findings reported herein advance this line of inquiry by establishing a specific mechanistic link between purified condensed tannins isolated from Platanus floral material and transcriptional downregulation of PCNA within a triple-negative breast cancer experimental framework (Hoque et al., 2025). The migratory potential of MDA-MB-231 cells was markedly compromised following exposure to the CT extract, extending the observed anti-tumor activity beyond mere proliferative inhibition. Quantitative analysis of wound-healing assays revealed that untreated control cells accomplished 80-95% scratch closure within a 24-hour interval, while cells subjected to 1.25 mg/mL CT treatment attained only 65-75% closure over an equivalent duration. Concurrent with this impaired migratory phenotype, MMP9 transcript levels declined by approximately 40% relative to controls. As a zinc-dependent endopeptidase, MMP9 functions in concert with MMP2 to proteolytically remodel extracellular matrix constituents, a process that is widely recognized as a prerequisite for tumor invasion and hematogenous dissemination (Egeblad & Werb, 2002; Wolosowicz et al., 2025). Elevated MMP9 expression has consistently been correlated with aggressive clinicopathological features and unfavorable outcomes in breast carcinoma patients (Mehner et al., 2014; Jiang & Li, 2021). The weakened wound closure with lower CT concentrations cannot be ascribed exclusively to a reduction in viable cell numbers arising from the cytostatic properties of the CTs. Rather, the targeted suppression of MMP9 transcription indicates a direct disruption of the cellular motility apparatus, operating independently of proliferative mechanisms (Rashid & Bardaweel, 2023; Lee

et al., 2024; Yang et al., 2024). This mechanistic distinction carries considerable biological significance, as it implies that CTs perturb at least two discrete oncogenic pathways simultaneously. Analogous anti-migratory properties have been reported for structurally related polyphenolic compounds; urolithin A, for example, demonstrated capacity to attend colorectal cancer dissemination, while suppression of matrix metalloproteinase expression constitutes a recurrent mechanistic feature attributable to plant-derived tannin fractions across multiple experimental systems (Zhao et al., 2018; Karakurt & Adali, 2016). The capacity of a single purified botanical extract to downregulate both PCNA expression and MMP9 activity necessitates consideration of a unifying upstream regulatory mechanism. Among the most biochemically tenable candidates is the NF-κB transcriptional signaling axis, a well-documented regulator of gene networks governing cellular survival, inflammatory response, and metastatic dissemination, including the transcriptional activation of MMP9 (Rashid & Bardaweel, 2023). Alternatively, perturbation of growth factor receptor-mediated signaling or dysregulation within the PI3K/Akt transduction cascade warrants equal consideration (Barry & McNabb, 1999; Khalid et al., 2021). These mechanistic propositions find conceptual support in the well-established protein-binding affinity of tannin compounds, a property that may impair enzymatic function and interrupt downstream intracellular signal propagation (Lee et al., 2024). It merits emphasis that earlier reports implicating tannins in carcinogenic activity have been substantially reattributed to confounding constituents inherent to crude extract preparations; more rigorous subsequent analyses affirm the chemopreventive and therapeutically relevant properties of isolated polyphenolic fractions (Chung et al., 1998). The biological efficacy of condensed tannins (CTs) is further governed by structural parameters, notably degree of polymerization and subunit configuration, underscoring the indispensability of thorough extract characterization prior to biological evaluation (Brown et al., 2017). In the present investigation, sequential application of gel filtration chromatography and thiolytic degradation yielded a structurally defined, highly purified CT fraction, thereby reinforcing the interpretive validity of the observed biological outcomes. Furthermore, the cytotoxic activity documented at elevated concentrations (2.5 and 5.0 mg/mL) aligns congruently with previously reported findings concerning CT-mediated effects on MDA-MB-231 cells, collectively substantiating the functional bioactivity of the extract under investigation (Al-Bedhawi et al., 2024; Karin, 2006; Haslam, 1996). Purified condensed tannins isolated from Platanus flowers demonstrate significant anti-malignant activity against MDA-MB-231 triple-negative breast cancer cells. At a sub-cytotoxic concentration, these compounds operate through a dual mechanism, simultaneously suppressing cellular proliferation and inhibiting migration. The concurrent downregulation of PCNA and MMP9 provides a compelling molecular basis for each of these effects, respectively. Collectively, these findings reinforce the growing body of evidence supporting plant-derived polyphenols as serious candidates in anti-cancer pharmaceutical development, particularly for aggressive and therapeutically challenging subtypes such as triple-negative breast cancer. Future investigations should prioritize the

structural characterization of the specific proanthocyanidin constituents responsible for driving these biological effects, as well as the delineation of the precise intracellular signaling pathways through which they exert their anti-tumor activity.

**Conclusions:** Condensed tannins from Platanus flowers purified, inhibit growth and migration of triple-negative breast cancer MDA-MB-231 cells by identifying a dual mechanism at sub-lethal concentrations. These tannins delay cell cycle progression, with a doubling time extended from 18 hours to 22 hours at 1.25 mg/mL, correlating with a 65% reduction in PCNA transcript levels. As PCNA is an essential cofactor for DNA replication, its knockdown results in physical cell cycle arrest. The extract also inhibits the migratory capacity of these cells; in wound healing assays, control cells achieve 95% closure within 24 hours compared to only 75% for cells treated with tannins. This is mediated through a 40% reduction in MMP9 levels, which is an enzyme responsible for degrading extracellular matrix components that facilitate cellular motility and invasion. Thus, these results are relevant as they demonstrate that the extract simultaneously targets two different oncogenic pathways: growth by targeting PCNA and migration by inhibiting MMP9. This supports the notion that botanical extracts could serve as starting points for pharmaceutical development toward aggressive cancer subtypes. Further studies need to elucidate the precise structural constituents of these tannins and the specific signaling axes such as NF-κB that they inhibit.

**Author contributions**

Mohammed Abdalmalek Ali Al-Bedhawi: Investigation, Formal analysis, Validation, Writing - original draft. Rami Altameemi: Data curation, Formal analysis, Validation, Writing - original draft. Sara Mahdi AL-Lami: Conceptualization, Methodology, Supervision, Writing - review & editing. Mohammed Munis Dakheel: Data curation, Formal analysis, Validation, Writing - original draft. Safaa Ehssan Atta: Resources, Validation.

**Data availability statement**

The data contributing to the findings of this study are available from the investigating researcher upon request.

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**Ethical considerations**

The authors avoided data fabrication, falsification, plagiarism, and misconduct.

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**Conflict of interest**

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# تأثیر تانن‌های متراکم خالص‌شده گل‌های درخت چنار بر فعالیت‌های بدخیم سلول‌های سرطان پستان

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## چکیده

**هدف:** گل‌های درخت چنار (*Platanus*) سرشار از تانن‌های متراکم (Condensed Tannins; CTs) هستند که دارای ویژگی‌های زیست‌فعال متعددی گزارش شده‌اند. با این حال، اثرات ضدسرطانی اختصاصی این ترکیبات بر سلول‌های سرطان پستان سه‌گانه منفی هنوز به خوبی شناخته نشده است. هدف پژوهش، ارزیابی اثرات وابسته به دوز این ترکیبات بر تکثیر سلولی، مهاجرت سلولی و بیان ژن‌های کلیدی مرتبط با بدخیمی (PCNA و MMP9) در سلول‌های سرطان پستان MDA-MB-231 بود.

**مواد و روش‌ها:** سلول‌های MDA-MB-231 با غلظت‌های متوالی تانن‌های متراکم خالص‌شده گل چنار (۱/۲۵، ۲/۵ و ۵ میلی‌گرم بر میلی‌لیتر) تیمار شدند. تکثیر سلولی از طریق محاسبه زمان دوبرابر شدن سلول‌ها با استفاده از میکروسکوپی تایم‌لپس (Time-lapse)و رهگیری تک‌سلولی توسط نرم‌افزار ImageJ ارزیابی شد. مهاجرت سلولی با استفاده از آزمون ترمیم زخم (Wound-healing assay)مورد بررسی قرار گرفت. میزان بیان ژن‌های آنتی‌ژن هسته‌ای سلول‌های در حال تکثیر (PCNA) و متریکس متالوپپتیداز ۹ (MMP9) به روش RT-qPCR اندازه‌گیری شد. نرخ تقسیم سلولی و مهاجرت ناشی از ترمیم زخم

به‌طور مداوم با استفاده از سامانه تصویربرداری زمان‌گریز Axio Imager پایش گردید. تحلیل‌های آماری با نرم‌افزارGraphPad Prism انجام شد.

**یافته‌ها**

تحلیل‌های تایم‌لپس پاسخ وابسته به دوز آشکاری را نشان دادند. غلظت‌های بالاتر تانن‌های متراکم (۲/۵ و ۵ میلی‌گرم بر میلی‌لیتر) موجب مرگ کامل سلول‌ها در ساعت نخست تیمار شدند. تیمار با غلظت ۱/۲۵ میلی‌گرم بر میلی‌لیتر بدون ایجاد سمیت حاد، اثر سیتواستاتیک (مهارکننده رشد سلولی) قوی ایجاد کرد و زمان دوبرابر شدن سلول‌ها را به‌طور معنی‌داری از حدود ۱۸ ساعت در گروه شاهد به حدود ۲۲ ساعت افزایش داد ($P<0.05$). نتایج آزمون ترمیم زخم نیز کاهش معنی‌دار بسته‌شدن زخم را پس از ۲۴ ساعت نشان داد؛ به‌طوری که میزان ترمیم زخم در گروه‌های تیمارشده ۶۵ تا ۷۵ درصد بود، در حالی که این مقدار در گروه شاهد بین ۸۰ تا ۹۵ درصد مشاهده شد ($P<0.05$). بررسی‌های مولکولی نشان داد که تیمار با ۱/۲۵ میلی‌گرم بر میلی‌لیتر تانن متراکم، بیان ژن PCNAرا ۶۵ درصد و بیان ژن MMP9 را ۴۰ درصد کاهش می‌دهد. الگوی تغییرات بیان ژنی مشاهده‌شده کاملاً با نتایج آزمون‌های تکثیر و مهاجرت سلولی همخوانی داشت.

**نتیجه‌گیری:** تانن‌های متراکم خالص‌شده جداشده از گل‌های چنار، فعالیت ضدبدخیمی قابل‌توجهی علیه سلول‌های سرطان پستان سه‌گانه منفی MDA-MB-231 نشان دادند. این اثر از طریق دو سازوکار اصلی اعمال می‌شود: مهار تکثیر سلولی از طریق کاهش بیان ژن PCNA و مهار مهاجرت سلولی از طریق کاهش بیان ژن MMP9. یافته‌های این پژوهش نشان می‌دهد که تانن‌های متراکم گل چنار می‌توانند به‌عنوان گزینه‌های امیدوارکننده‌ای برای توسعه داروهای ضدسرطان مورد توجه قرار گیرند.

**کلمات کلیدی:** تانن‌های متراکم (CTs)، تصویربرداری زمان‌گریز (Time-lapse)، درخت چنار، MDA، PCNA

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