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ORIGINAL RESEARCH

The Effects of *Althaea officinalis* L. Extract on Cell Viability, Invasion, and Apoptosis in Prostate Cancer

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ABSTRACT

This study aims to evaluate the cytotoxic effects of methanol (AOME) and ethyl acetate (AOEE) extracts obtained from the flowers of *Althaea officinalis* L., which is known for its high antioxidant capacity, on prostate cancer cell lines (LNCaP and PC3), as well as their inhibitory effects on cell migration and invasion, and their influence on pro-apoptotic gene expression. Cell viability was assessed using the MTT assay, while cell migration and invasion were analyzed using the scratch assay. Changes in pro-apoptotic gene expression were evaluated by real-time PCR (RT-qPCR), as determined at the IC_{50} concentrations obtained from cytotoxicity tests. The results showed that AOME and AOEE induced dose-dependent cytotoxicity in both cell lines. The IC_{50} values were found to be 1.1×10^{-3} µg/mL (AOME) and 11×10^{-5} µg/mL (AOEE) for PC3 cells, and 0.21×10^{-3} µg/mL (AOME) and 0.45×10^{-3} µg/mL (AOEE) for LNCaP cells. Scratch assay analyses demonstrated that both extracts significantly inhibited cell migration and invasion compared to the control group. Additionally, RT-qPCR analyses revealed that pro-apoptotic gene expression was significantly increased, and anti-apoptotic gene expression was significantly decreased in treated cells ($p<0.05$). These findings suggest that A. officinalis extracts possess properties that reduce cell viability, inhibit migration and invasion, and promote apoptosis in prostate cancer cells, indicating that the extract may have therapeutic potential against prostate cancer and warrant further validation through comprehensive molecular investigations.

Keywords: ***Althaea officinalis.*** **Apoptosis. Prostate cancer. Cell migration.**

Althaea officinalis **L. Ekstraktının Prostat Kanserinde Hücre Canlılığı, İnvazyon ve Apoptoz Üzerindeki Etkileri**

ÖZET

Bu çalışmanın amacı, yüksek antioksidan kapasitesi ile bilinen *Althaea officinalis* L. çiçeklerinden elde edilen metanol (AOME) ve etil asetat (AOEE) ekstrelerinin prostat kanseri hücre hatları (LNCaP ve PC3) üzerindeki sitotoksik etkilerini, hücre göçü ve invazyonu üzerindeki inhibe edici etkilerini ve pro-apoptotik gen ekspresyonuna etkilerini değerlendirmektir. Hücre canlılığı MTT testi ile değerlendirilmiş, hücre göçü ve invazyonu scratch testi ile analiz edilmiştir. Pro-apoptotik gen ekspresyonundaki değişiklikler, sitotoksisite testlerinden elde edilen IC_{50} konsantrasyonlarında değerlendirilmiştir. Sonuçlar, AOME ve AOEE'nin her iki hücre hattında doz bağımlı sitotoksisite indüklediğini göstermiştir. IC_{50} değerleri PC3 hücreleri için $1{,}1\times10^{-3}$ µg/mL (AOME) ve 11×10^{-5} µg/mL (AOEE), LNCaP hücreleri için ise $0{,}21\times10^{-3}$ µg/mL (AOME) ve $0{,}45\times10^{-3}$ µg/mL (AOEE) olarak bulunmuştur. Scratch testi analizleri, her iki ekstrenin de hücre göçü ve invazyonunu kontrol grubuna göre anlamlı şekilde engellediğini göstermiştir. Ayrıca, RT-qPCR analizleri, tedavi edilen hücrelerde pro-apoptotik gen ekspresyonunun anlamlı şekilde arttığını ve anti-apoptotik gen ekspresyonunun anlamlı şekilde azaldığını ortaya koymuştur ($p<0{,}05$). Bu bulgular, *A. officinalis* ekstrelerinin hücre canlılığını azalttığını, göç ve invazyonu engellediğini ve apoptosis'i teşvik ettiğini göstermekte olup, ekstrenin prostat kanseri üzerinde terapötik potansiyele sahip olabileceğini ve bu durumun kapsamlı moleküler çalışmalarla doğrulanması gerektiğini düşündürmektedir.

Anahtar Kelimeler: ***Althaea officinalis.*** **Apoptoz. Prostat kanseri. Hücre göçü.**

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Following cardiovascular diseases, cancer is the second most fatal disease worldwide. Malignant diseases occur when the normal process of cell division is disrupted. A decrease in apoptosis and an increase in cell proliferation lead to the formation of malignant cells[1]. While prostate cancer is the fourth most common type of cancer worldwide, it ranks second in cancer-related deaths after lung cancer. Additionally, it is the most common cancer in males after skin cancer[2]. Prostate cancer can be asymptomatic at an early stage, although the most

common complaint is increased frequency of urination and nocturia, which may result from prostate hypertrophy. Bone metastasis is an important problem in prostate cancer[3]. Factors contributing to prostate cancer include permanent changes in DNA due to DNA damage, disruption of control points in cell division, and hypoxic conditions[1].

As in many types of cancer, several treatment methods are used in prostate cancer, including chemotherapy, radiotherapy, and surgical resection, although these methods have significant drawbacks, such as drug resistance in cancer cells and toxicity in normal cells. Therefore, new pharmacological approaches are needed to address these challenges. Plant-based drugs are known to have important effects, especially in cancer, as they can inactivate cancer-initiating enzymes and hormones, activate DNA repair mechanisms, stimulate the production of cancer-inhibiting enzymes, induce cell death, halt the cell division cycle, and enhance the immune system. Approximately 70% of cancer drugs used in the pharmaceutical industry are obtained from natural sources. Many herbal anticancer agents, such as taxol, vinblastine, vincristine, topotecan, and irinotecan, are in clinical use worldwide[4].

Natural products derived from plants provide a crucial resource for drug development, as medicines produced from natural products constitute 28% of all medicines. Other drugs have been developed from natural products, particularly through the synthetic production of compounds found in plants[5]. *Althaea officinalis* L. (*A. officinalis*) is a plant native to Asia, Europe, and the United States. It has been traditionally used to treat oral and pharyngeal mucosal irritation, dry cough, mild gastritis, skin burns, insect bites, cold, and gastrointestinal and urinary tract complaints. Studies report that it exhibits anti-inflammatory, immunostimulant, and phagocytic activity and is effective against tooth and gum abscesses, in addition to possessing antibacterial properties[6]. In the study conducted by Al-Snafi et al. (2013), many compounds were identified in different parts of *A. officinalis*, including 11% pectins, 25–35% starch, 10% mono- and disaccharide sucrose, 5% mucilage, flavonoids (Hypolaetin-8-glucoside, isoorientin, kaempferol, caffeic acid, p-coumaric acid), coumarins, scopoletin, phytosterols, tannins, asparagine, and various amino acids[7].

Free oxygen radicals are a key factor in cancer development, and *A. officinalis* is an important source of antioxidants. Studies have shown that ethanol extracts of *A. officinalis* can effectively eliminate free oxygen radicals, although the specific compounds responsible for this activity are currently unclear[6]. The literature also reports that *A. officinalis* extract may be considered a potential anticancer agent[8].

In this context, the present study, conducted for the first time in the literature, aims to evaluate the effects of methanol (AOME) and ethyl acetate (AOEE) extracts derived from *A. officinalis* flowers on cell proliferation, invasion, and apoptosis in PC3 and LNCaP prostate cancer cell lines. The findings from this study are expected to contribute to a better understanding of the anticancer potential of natural products and support the development of new therapeutic approaches.

Material and Method

Preparation of plant extracts: Preparation of AOME and AOEE extracts from A. officinalis

The plant material used in the study was obtained from a local herbal source, with collection confirmed in Balıkesir, Türkiye. The powder of dried *A. officinalis* flowers was weighed as 15gg each and placed in separate glass bottles. The weighed powders were combined with pure methanol (Lichrosolv, Germany) or pure ethyl acetate (Sigma-Aldrich, USA) and stirred at 40°C for 72 hours using a magnetic stirrer. The resulting suspensions were filtered through Macherey-Nagel filter paper (125 mm, Germany) and concentrated using a rotary evaporator (Heidolph, Germany) at 50 mbar and 40°C until dry. For the preparation of stock solutions, the extracts were reweighed and dissolved in 5 mL of deionized water, yielding final stock concentrations of 156.7 mg/mL (AOME) and 37.6 mg/mL (AOEE). The solutions were sterilized using 0.22 µm filters and stored at −20°C.

HPLC analysis

In this study, HPLC analysis was performed to identify the chemical components of AOME and AOEE extracts obtained from the flowers of *A. officinalis*, with the analyses conducted at the Balıkesir University Science and Technology Application and Research Center (BÜBTAM). The HPLC method was developed using a Shimadzu RF-20A liquid chromatography system, which was equipped with an LC-10AT pump, a CBM-20A lite system controller, an SPD-M20A DAD detector capable of measuring in the 190–800 nm wavelength range, a CTO-10ASVP column oven, a DGU-20A5R degasser, and a SIL-20AHT auto-injector. For the analysis, a Prontosil Hypersorb ODS C18 reversed-phase column (150 × 4.6 mm, 3 µm), protected by a guard column, was employed. The mobile phase consisted of 0.1% formic acid in water (A) and acetonitrile (B), and the following gradient elution program was applied: 0–10 min, 13–16% B; 10–11 min, 16–17% B; 11–25 min, 17% B; 25–55 min, 17–65% B, with the flow rate set at 1.0 mL/min and the

injection volume at 5 µL. The final solution was filtered through a 0.22 µm pore-size membrane filter to obtain the reference solution.

Cell lines

The PC3 (Cat. No. CRL-1435) and LNCaP (Cat. No. CRL-1740) human prostate cancer cell lines were obtained from cell stocks originating from ATCC (American Type Culture Collection). PC3 cells, an androgen-independent human prostate cancer cell line, were cultured in F12 medium (Gibco, Germany) supplemented with 10% Fetal Calf Serum (FCS) in 75 cm^2 flasks at 37°C under 5% CO_2 atmospheric conditions. LNCaP cells, an androgen-sensitive human prostate cancer cell line, were cultured in RPMI 1640 medium (Gibco, Germany) supplemented with 10% FCS in 75 cm^2 flasks at 37°C under 5% CO_2 atmospheric conditions. The FCS used was heat-inactivated at 55°C for one hour, filtered through a 0.22 µm filter, stored at −20°C, and used as required.

Establishing cytotoxicity experiments

The cells were counted in triplicate using a Neubauer chamber and then seeded into 96-well plates at a density of 5×10^3 cells per well with 200 µL of medium, which was appropriate for each cell line. The cells were incubated for 24 hours at 37°C under 5% CO_2 atmospheric conditions. Control groups with no treatment and groups treated with different doses of AOME and AOEE were prepared, and the cells were incubated for 24, 48, and 72 hours. The experiments were conducted with at least three replicates. For the cytotoxicity (MTT) assay, at the end of the 24, 48, and 72-hour incubation periods, 20 µL of MTT solution at a concentration of 0.50 mg/mL was added to each well containing cells. The cells were incubated for 4 hours at 37°C under 5% CO_2. At the end of the incubation, the medium was removed, and isopropanol containing 0.0004 M HCl was added to the wells. Afterward, absorbance was measured at 550 nm using a spectrophotometer (Thermo Scientific Multiskan Sky Microplate Spectrophotometer with Touch Screen + µDrop Plate, USA).

Cell migration analysis

LNCaP and PC3 cells were seeded into two 12-well plates at a density of 5×10^5 cells per well and incubated overnight. Scratches were made in each well using a 200 µL pipette tip, and cell debris was removed with PBS before fresh medium was added. Groups treated with the IC50 doses of AOME and AOEE, and control groups with no treatment, were photographed at 0, 24, and 48 hours. The images were analyzed using the ImageJ program. The experiments were performed in triplicate, and the invasion ability of the cells was measured by determining the percentage of area and occupancy.

RNA isolation and cDNA synthesis

RNA isolation was performed according to the protocol of the Thermo Scientific GeneJET RNA Purification Kit (USA). The concentration of total RNA samples was determined by spectrophotometric measurements, and the isolated RNA was stored at -80 °C until further use. cDNA synthesis was carried out using the Thermo Scientific RevertAid First Strand cDNA Synthesis Kit (USA), following the manufacturer's instructions. For each cell line, 1 µg of total RNA was mixed with 1 µL of oligo(dT)18 primer and RNase-free water to a total volume of 20 µL and incubated at 65 °C for 5 minutes. Subsequently, 4 µL of 5x reaction buffer, 1 µL of RNase inhibitor (20 U/µL), 2 µL of 10 mM dNTP mix, and 1 µL of RevertAid M-MuLV Reverse Transcriptase (200 U/µL) were added to the mixture. The reaction was incubated at 42 °C for 60 minutes, followed by enzyme inactivation at 70 °C for 5 minutes. The synthesized cDNA was stored at -20 °C until further use.

Real-Time PCR analysis

RT-qPCR was performed using the WizPure RT-PCR 2X Master Kit (Wizbiosolutions, South Korea) according to the manufacturer's protocol. Gene expression levels of Bcl-2, Bcl-xL, Bak, and Bax, as well as the housekeeping gene Hβ-2, were analyzed using specific primers, as listed in Table I. For each reaction, the PCR mixture was prepared to a total volume of 20 µL, containing 1 µL of cDNA template, 10 µL of RT-PCR 2x Master Mix, 1 µL of 10 µM forward primer, 1 µL of 10 µM reverse primer, and 7 µL of RNase-free water. Amplification was carried out using the Applied Biosystems 7500 Real-Time PCR System (Foster City, CA, USA) under the following cycling conditions: initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 20 seconds, and extension at 72°C for 1 minute. A final extension was performed at 72°C for 5 minutes. Gene expression levels were determined using the $2^{-\Delta\Delta Ct}$ method, comparing treated samples to the control group[9].

Statistical analysis

Statistical analyses of the data obtained from cytotoxicity, mRNA expression, and cell migration assays were performed using Minitab 14 software. The normality of the data was assessed using the Shapiro-Wilk test, and one-way analysis of variance (ANOVA) was applied to determine differences between groups. When significant differences were observed, Tukey's post-hoc test was conducted for pairwise comparisons. All data are presented as mean ± standard deviation (SD), and p-values <0.05 were considered statistically significant.

Table I. Primer sequences used for Real-time PCR analysis of target genes

Name of gene	Primer sequence (5'– 3')
Bcl-2 Forward	CTGCACCTGACGCCCTTCACC
Bcl-2 Reverse	CACATGACCCCACCGAACTCAAAGA
Bcl-xL Forward	GATCCCCATGGCAGCAGTAAAGCAAG
Bcl-xL Reverse	CCCCATCCCGGAAGAGTTCATCTACT
Bax Forward	TTTGCTTCAGGGTTTCATC
Bax Reverse	TCCTCTGCAGCTCCATGTTA
Bak Forward	ACCAGCCTGTTTGAGAGTGG
Bak Reverse	AGTGATGCAGCATGAAGTCG
Hβ-2 Forward	CCTGACTGACTACCTCATGAAGATCCTC
Hβ-2 Reverse	CGTAGCACAGCTTCTCCTTAATGTCAC

Results

HPLC analysis results

The presence and quantities of phenolic compounds in the AOME and AOEE extracts of *A. officinalis* flowers were analyzed using the HPLC method. Six main phenolic compounds—vanillic acid, caffeic acid, ferulic acid, p-coumaric acid, rutin hydrate, and quercetin—were detected in both extracts, and the obtained retention times and concentration values are presented in Table II.

Table II. HPLC analysis results of AOME and AOEE extracts of *A. officinalis* flower

Sample	Compound Name	Concentration (ng/µL)	Retention Time (min)
AOME	Vanillic acid	4.840	9.502
	Caffeic acid	4.190	9.902
	p-coumaric acid	*	*
	Ferulic acid	38.700	15.710
	Rutin hydrate	*	*
	Quercetin	6.990	26.919
AOEE	Vanillic acid	0.989	9.665
	Caffeic acid	*	*
	p-coumaric acid	23.140	14.227
	Ferulic acid	0.404	16.085
	Rutin hydrate	8.600	17.129
	Quercetin	*	*

* Not detected by HPLC.

In the AOME extract, ferulic acid was detected at the highest concentration (38.700 ng/µL), while quercetin and vanillic acid were found at 6.990 ng/µL and 4.840 ng/µL, respectively. Caffeic acid was determined at 4.190 ng/µL, whereas p-coumaric acid and rutin hydrate were not detected in the AOME extract, indicating that this extract is particularly rich in ferulic acid.

In the AOEE extract, p-coumaric acid (23.140 ng/µL) and rutin hydrate (8.600 ng/µL) were detected at the highest levels. Vanillic acid and ferulic acid were detected at concentrations of 0.989 ng/µL and 0.404 ng/µL, respectively. Caffeic acid and quercetin were not detected in the AOEE extract, showing that AOEE is richer in p-coumaric acid and rutin hydrate compared to AOME.

The antioxidant and anticancer properties of phenolic compounds are extensively supported in the literature. Differences in the compound profiles of the AOME and AOEE extracts may explain the diversity of the biological activities of *A. officinalis*. In particular, ferulic acid and quercetin are known for their potential to inhibit proliferation and activate apoptotic mechanisms in cancer cells. Therefore, it is considered that the compound compositions of both extracts contribute differently to their anticancer potential.

In vitro cytotoxicity profiles of extracts using the MTT assay

In LNCaP cells treated with AOME, cell viability changed with increasing doses at 24 hours; it was 89.2% at 0.5×10^{-3} µg/mL, 78.1% at 0.75×10^{-3} µg/mL, 85.8% at 1×10^{-3} µg/mL, 49.7% at 1.5×10^{-3} µg/mL, and 78.9% at 1.75×10^{-3} µg/mL. At 48 hours, viability decreased markedly, with the lowest value observed at 26.8% at 1.5×10^{-3} µg/mL, while other doses ranged between 30.5% and 55.8%. At 72 hours, cell viability was below 20% and independent of dose. In LNCaP cells treated with AOEE, cell viability at 24 hours was 58.6% at 6.25×10^{-5} µg/mL, 51.9% at 12.5×10^{-5} µg/mL, 59.5% at 25×10^{-5} µg/mL, 44.8% at 50×10^{-5} µg/mL, and 36.6% at 62.5×10^{-5} µg/mL. At 48 hours, viability varied independently of dose, with values of 61.3% at 6.25×10^{-5} µg/mL, 72.8% at 12.5×10^{-5} µg/mL, 53.2% at 25×10^{-5} µg/mL, 35.1% at 50×10^{-5} µg/mL, and 23.9% at 62.5×10^{-5} µg/mL. At 72 hours, cell viability decreased in a dose-dependent manner, ranging from 70% to 20% (Figure 1).

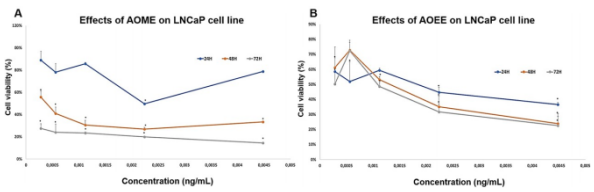

Figure 1:
Cell viability of LNCaP cells after treatment with AOME and AOEE. Cells were treated with different concentrations of AOME ($0.5–1.75\times10^{-3}$ µg/mL) or AOEE ($6.25–62.5\times10^{-5}$ µg/mL), and cell viability was assessed at 24, 48, and 72 hours using the MTT assay, with data presented as the mean ± standard deviation of three independent experiments and statistical analysis performed using ANOVA, with significance set at $p < 0.05$.

In PC3 cells treated with AOME, cell viability at 24 hours was 86.9% at 0.5×10^{-3} µg/mL, 61.5% at 0.75×10^{-3} µg/mL, 47.2% at 1×10^{-3} µg/mL, 53.4% at 1.5×10^{-3} µg/mL, and 51.6% at 1.75×10^{-3} µg/mL. At 48 hours, viability generally decreased, with values of 85.5% at 0.5×10^{-3} µg/mL, 51.0% at 0.75×10^{-3} µg/mL, 44.9% at 1×10^{-3} µg/mL, 57.0% at 1.5×10^{-3} µg/mL, and 42.9% at 1.75×10^{-3} µg/mL. At 72 hours, cell viability was below 40% and independent of dose. In PC3 cells treated with AOEE, viability at 24 hours ranged from 32.1% to 75.2% at low and medium concentrations ($6.25–25\times10^{-5}$ µg/mL), while at higher concentrations (50 and 62.5×10^{-5} µg/mL) it was 20.6% and 16.9%, respectively. At 48 hours, viability increased at low and medium concentrations (104–113.9%) but decreased at higher concentrations (15.4% and 10.4%). At 72 hours, cell viability resembled that observed at 24 and 48 hours, with a marked decrease at high doses (Figure 2).

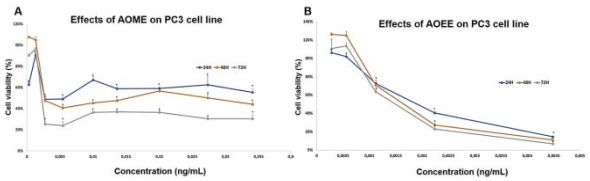

Figure 2:
Cell viability of PC3 cells after treatment with AOME and AOEE. Cells were treated with different concentrations of AOME (0.5–1.75×10⁻³ µg/mL) or AOEE (6.25–62.5×10⁻⁵ µg/mL), and cell viability was assessed at 24, 48, and 72 hours using the MTT assay, with data presented as the mean ± standard deviation of three independent experiments and statistical analysis performed using ANOVA, with significance set at $p < 0.05$.

Overall, both extracts exhibited anti-proliferative effects in LNCaP and PC3 cells. Because cell viability decreased markedly at 72 hours in both cell lines, only the 24- and 48-hour time points were used in subsequent experiments. The effects varied depending on cell type, dose, and exposure time, with AOEE exhibiting stronger dose- and time-dependent cytotoxicity in LNCaP cells. In contrast, low and medium doses in PC3 cells resulted in increased viability.

Effect of AOME and AOEE on cell migration assessed by scratch assay

The effects of AOEE and AOME on cell migration in LNCaP and PC3 cell lines were evaluated at doses corresponding to their IC_{50} values (LNCaP: AOEE 0.45×10^{-3} µg/mL, AOME 0.21×10^{-3} µg/mL; PC3: AOEE 1.1×10^{-3} µg/mL, AOME 11×10^{-5} µg/mL) and at 0, 24, and 48 hours, and were visualized microscopically, with the results indicating that both extracts reduced migration in a time- and dose-dependent manner (Figure 3).

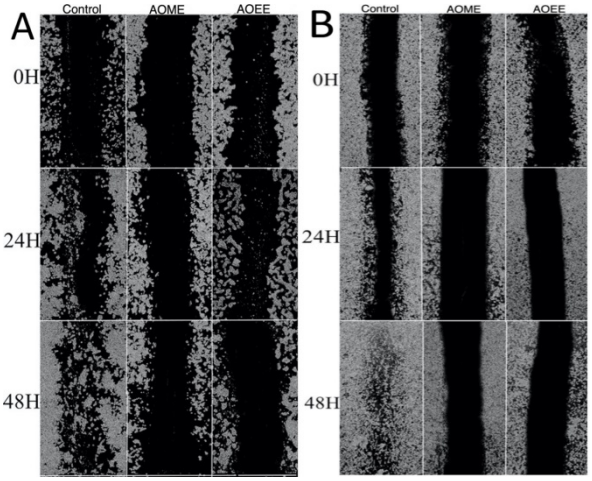

Figure 3:
Scratch assay images showing the effects of AOME and AOEE extracts on cell migration. (A) Migration analysis of LNCaP cells, and (B) migration analysis of PC3 cells, with images captured and analyzed using ImageJ software at 0, 24, and 48 hours. All experiments were performed in triplicate.

In LNCaP cells, AOME inhibited migration by 85.72% at 24 hours and by 80.71% at 48 hours, whereas AOEE was effective only at 48 hours, reducing migration by 86% (Figure 4). In PC3 cells, AOME decreased migration by 90% at 24 hours and by 75% at 48 hours, while AOEE showed no significant effect at 24 hours but reduced migration by 78.48% at 48 hours (Figure 5).

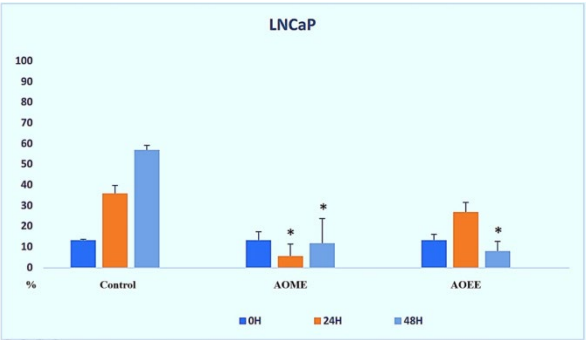

Figure 4:
Effects of AOME and AOEE on the migration of LNCaP cells. Cells were treated with doses corresponding to their IC_{50} values (AOEE 0.45×10^{-3} µg/mL, AOME 0.21×10^{-3} µg/mL). Migration was assessed at 0, 24, and 48 hours, with AOME significantly inhibiting migration by 85.72% at 24 hours and by 80.71% at 48 hours, while AOEE reduced migration only at 48 hours by 86%. Data are presented as the mean ± standard deviation of three independent experiments, and statistical analysis was performed using ANOVA, with significance indicated as $*p < 0.05$.

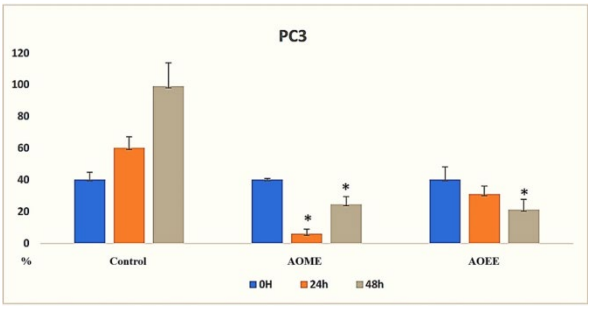

Figure 5:

*Effects of AOME and AOEE on the migration of PC3 cells. Cells were treated with doses corresponding to their IC_{50} values (AOEE 1.1 × 10^{-3} µg/mL, AOME 11 × 10^{-5} µg/mL), and migration was assessed at 0, 24, and 48 hours, with AOME reducing migration by 90% at 24 hours and by 75% at 48 hours, while AOEE showed no significant effect at 24 hours but reduced migration by 78.48% at 48 hours. Data are presented as the mean ± standard deviation of three independent experiments, and statistical analysis was performed using ANOVA, with significance indicated as *p < 0.05.*

Gene Expression Profiles Revealed by Real-Time PCR

RT-qPCR analysis in LNCaP and PC3 cells revealed the effects of AOME and AOEE on apoptosis-related gene (Bcl-2, Bcl-xL, Bak, Bax) mRNA levels, with relative expression normalized to the control group.

In LNCaP cells, following treatment with AOME and AOEE, Bcl-2 expression was measured as 0.83 in the AOME group and 0.63 in the AOEE group. Similarly, Bcl-xL expression decreased to 0.52 in both the AOME and AOEE groups. In contrast, Bak expression increased 1.75-fold in the AOME group and 1.67-fold in the AOEE group. In contrast, Bax expression was elevated 3.15-fold in the AOME group and 3.20-fold in the AOEE group, indicating that AOME and AOEE extracts suppress anti-apoptotic gene expression while enhancing pro-apoptotic gene expression in LNCaP cells, thereby promoting apoptosis (Figure 6).

In PC3 cells, following AOME and AOEE treatments, Bcl-2 expression was 1.07-fold in the AOME group and 1.18-fold in the AOEE group. In contrast, Bcl-xL expression was 1.04-fold in the AOME group and 1.30-fold in the AOEE group, showing no significant changes compared to the control group. These data suggest that both extracts caused a slight increase in anti-apoptotic gene expression, although these increases may be of limited biological significance. In contrast, Bak expression increased 1.59-fold in the AOME group and 2.14-fold in the AOEE group, whereas Bax expression increased 2.06-fold and 2.19-fold in the AOME and AOEE groups, respectively. This indicates that both extracts exert a strong stimulatory effect, particularly on pro-apoptotic genes (Figure 7).

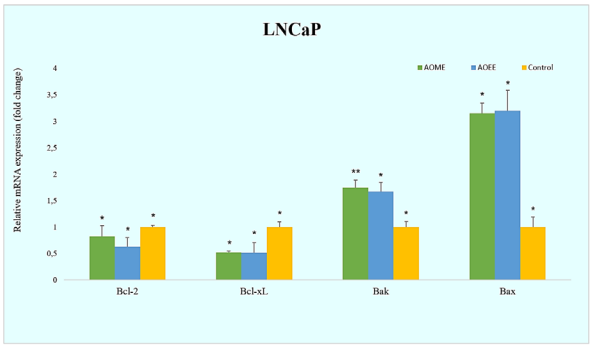

Figure 6:

*Effects of AOME and AOEE on apoptosis-related gene expression in LNCaP cells. The expression of anti-apoptotic genes (Bcl-2 and Bcl-xL) and pro-apoptotic genes (Bak and Bax) was determined, with both AOME and AOEE suppressing the expression of Bcl-2 and Bcl-xL while increasing the expression of Bak and Bax. Data are presented as the mean ± standard deviation of three independent experiments, and statistical analysis was performed using ANOVA, with significance indicated as *p < 0.05, **p < 0.01, and ***p < 0.001.*

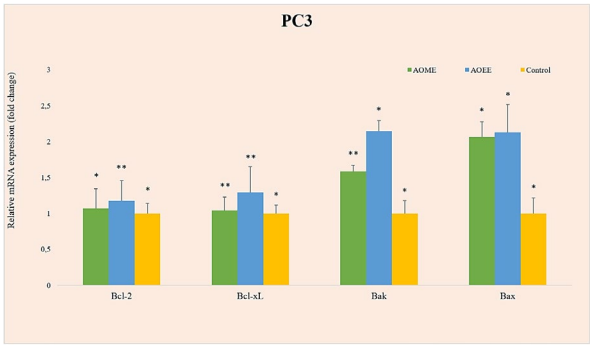

Figure 7:

*Effects of AOME and AOEE on apoptosis-related gene expression in PC3 cells. The expression of anti-apoptotic genes (Bcl-2 and Bcl-xL) showed slight increases in both AOME and AOEE groups without significant changes compared to the control. In contrast, the expression of pro-apoptotic genes (Bak and Bax) was markedly increased. Data are presented as the mean ± standard deviation of three independent experiments, and statistical analysis was performed using ANOVA, with significance indicated as *p < 0.05, **p < 0.01, and ***p < 0.001.*

Discussion and Conclusion

A. officinalis is considered a potential antitumor agent due to its high antioxidant content. Previous studies have reported that ethanol extracts of *A. officinalis* inhibit lipid peroxidation and exhibit biological activities, including free radical scavenging, superoxide anion neutralization, and metal chelation[10,

[11]. Flavonoids present in the flower extracts, including vitamin A, rutin, apigenin, isorhamnetin, scopoletin, coumarins, and kaempferol, have been identified as bioactive compounds with potential cancer-preventive effects[12,13]. The high flavonoid content in light-colored flowers supports their use in medicinal applications, and this approach was also adopted in the present study[14].

The biological effects of *A. officinalis* on in vitro cancer cell lines have been investigated in a limited number of studies. Ciobanu et al. (2019) indicated that extracts applied to Hep-2B liver cancer cells reduced cell viability[15]. Kadhum et al. (2021) reported that the crude extract and its flavonoid and phytosterol fractions exhibited dose-dependent cytotoxic effects on the AMJ13 breast cancer cell line, with the crude extract showing the highest cytotoxicity at an IC50 value of 25.18 µg/mL, inducing apoptosis with a potential comparable to doxorubicin[16]. Zhang et al. (2017) demonstrated that root extracts of *A. officinalis* exert both cytotoxic and antiproliferative effects on A549 lung cancer cells and that these effects can be modulated when combined with cisplatin[8]. In another study, Farhat et al. (2022) reported that aqueous flower extracts exerted cytotoxic effects on A549 (lung), HCT116 (colorectal, p53-negative and positive), MCF-7 (breast), and HeLa (cervical) cancer cell lines, with pronounced effects particularly in A549 cells[17].

The present study systematically evaluated, for the first time, the anti-proliferative and pro-apoptotic effects of *A. officinalis* AOME and AOEE extracts on PC3 and LNCaP cells. The findings indicate that the extracts inhibit proliferation and modulate apoptotic gene expression in a cell type-, dose-, and time-dependent manner. Specifically, a biphasic response profile was observed in PC3 cells, whereas a pronounced cytotoxic effect was detected in LNCaP cells, revealing cell type-specific biological activities of the extracts.

Apoptosis is defined as programmed cell death[18], and the Bcl-2 family genes regulate this process[19]. The anti-apoptotic (Bcl-2, Bcl-xL) and pro-apoptotic (Bax, Bak) members of this family are critical determinants of cell survival and death[20, 21, 22]. Bax and Bak act as key components that regulate apoptotic pathways through the mitochondrial axis[23]. In the present study, analysis of apoptosis-related genes revealed that in LNCaP cells, the anti-apoptotic genes Bcl-2 and Bcl-xL were significantly downregulated, whereas the pro-apoptotic genes Bak and Bax were markedly upregulated, indicating strong activation of the mitochondrial apoptotic pathway. In PC3 cells, anti-apoptotic genes showed limited upregulation, while pro-apoptotic genes were significantly upregulated, with the AOEE extract enhancing this effect.

The differential responses of LNCaP (androgen-dependent) and PC3 (androgen-independent) cells to the extracts reflect tumor heterogeneity observed in prostate cancer. These cell lines possess distinct genetic and epigenetic profiles, leading to differences in proliferation, apoptosis sensitivity, and response to signaling pathways[24,25]. AOEE and AOME extracts exhibited more pronounced cytotoxicity in LNCaP cells, whereas PC3 cells displayed dose- and time-dependent biphasic responses, highlighting the significance of tumor heterogeneity in shaping treatment responses and the need for personalized strategies based on specific cell subtypes[26].

The biphasic response observed in PC3 cells corresponds to the phenomenon of hormesis described in the literatüre[27]. Hormesis is characterized by low doses of stress-inducing agents activating adaptive cellular defense mechanisms, whereas high doses result in cellular damage. At low doses, the extracts are thought to support cell survival by activating antioxidant systems (e.g., Nrf2/HO-1 pathways), while at high doses, oxidative stress predominates, inducing intrinsic mitochondrial or caspase-mediated apoptotic pathways[28]. Furthermore, stress adaptation responses involving changes in cellular redox balance may trigger proliferative or pro-apoptotic processes through signaling pathways such as MAPK and JAK-STAT[29,30]. Therefore, the paradoxical effect observed in PC3 cells is considered a result of dose-dependent differential cellular responses, and future mechanistic studies investigating these pathways at the protein level would clarify the molecular basis of the biphasic effect.

Cell migration analyses demonstrated that AOEE and AOME extracts reduced cell migration and wound closure in both cell lines in a dose- and time-dependent manner, which is consistent with our cytotoxicity and gene expression findings. Because inhibition of cell migration is a critical indicator for metastasis prevention, these results suggest that *A. officinalis* may suppress tumor progression by promoting apoptosis and reducing metastatic potential[31, 32].

HPLC analysis findings revealed the phenolic compound profiles of AOME and AOEE extracts, showing that ferulic acid was found at elevated levels in AOME extract, whereas coumaric acid was detected at higher levels in AOEE extract. Ferulic acid exerts anticancer effects in PC3 cells by increasing the expression of apoptosis- and metastasis-related genes[34]. It has been demonstrated that p-coumaric acid can induce apoptotic effects in prostate cancer cells, particularly by targeting the active sites of proteins such as MAPK8 and STK3, thereby increasing reactive oxygen species (ROS) production and inducing apoptosis[34]. Rutin hydrate has been reported to reduce cell viability in LNCaP cells in a dose-

dependent manner and to increase apoptosis rates as assessed by flow cytometry[34]. Vanilic acid has been associated with apoptosis and cell cycle regulation in prostate cancer cells as an HDAC3 inhibitor[33]. Caffeic acid phenethyl ester (CAPE) produces anticancer effects in PC3 cells by inhibiting EGFR/Akt signaling pathways, while quercetin exhibits anticancer properties by suppressing signaling molecules related to invasion, migration, and cell survival properties[35, 36]. Considering the differences in the phenolic compound profiles of the extracts and the potential variability in their biological activities, AOME and AOEE extracts were investigated independently, suggesting that future studies should examine the specific biological effects of each compound in its purified form to improve reproducibility and comparability with other studies.

Overall, the present study demonstrates that *A. officinalis* AOME and AOEE extracts can induce apoptosis and modulate proliferation and migration in prostate cancer cells. However, the study has several limitations. No standard chemotherapeutic agent (e.g., doxorubicin or cisplatin) was included as a positive control, limiting direct comparison with commonly used reference treatments. Additionally, only prostate cancer cell lines were studied, and normal prostate epithelial cell lines (e.g., RWPE-1 or PNT2) were not included, preventing a more comprehensive assessment of selective cytotoxicity. Future confirmatory studies incorporating both standard chemotherapeutics and normal cell lines would strengthen the clinical relevance and generalizability of the findings.

Although apoptosis-related gene mRNA expression profiles were analyzed, transcriptional changes do not always correspond directly to protein-level outcomes. Therefore, the results provide limited insight into the full activation of cellular signaling pathways. The absence of protein-level analysis of intrinsic (mitochondrial), extrinsic (death receptor), p53-mediated, and endoplasmic reticulum stress pathways, as well as MAPK and JAK-STAT pathways, which are critical for apoptosis and cell cycle regulation, limits the mechanistic interpretation. In particular, the lack of assessment of Bcl-2 family proteins (Bcl-2, Bax, Bak) and key components of the caspase cascade (e.g., cleaved caspase-3) prevents definitive conclusions regarding pathway activation. Future studies should validate these gene products and signaling pathways at the translational level using Western blot or immunoblot techniques, which would provide evidence of functional pathway activation in addition to transcriptional changes, thereby enhancing scientific reliability.

The findings were demonstrated only in vitro, providing mechanistic insights at the cellular level but not fully reflecting in vivo conditions such as the tumor microenvironment, immune interactions, angiogenesis, and pharmacokinetic/pharmacodynamic parameters. Therefore, the biological and clinical generalizability of the current results is limited. Future studies using appropriate prostate cancer animal models to evaluate the effects of *A. officinalis* extracts on tumor growth, apoptosis induction, metastatic potential, and therapeutic efficacy would allow for a more comprehensive understanding and in vivo validation, enabling the assessment of these extracts as potential adjuvant agents in prostate cancer therapy.

HPLC analysis in the present study identified only four phenolic compounds, indicating that the chemical diversity and potential biological effects of the extracts may not be fully captured. Therefore, future studies should employ advanced analytical techniques, such as LC–MS/MS, to characterize the phenolic compound diversity more comprehensively in A. officinalis extracts and elucidate the molecular mechanisms underlying the observed biological effects.

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References

1. Kosova F, Arı Z. Prostat kanseri ve apoptozis ilişkisi. J Clin Exp Invest. 2011;2(1):124–131.
2. Peng X, Zhuang DD, Guo QS. Induction of S phase arrest and apoptosis by ethyl acetate extract from *Tetrastigma hemsleyanum* in human hepatoma HepG2 cells. Tumour Biol. 2015;36(4):2541–2550.
3. Rawla P. Epidemiology of prostate cancer. World J Oncol. 2019;10(2):63–89.
4. Kılınç K, Demir S, Turan İ, Menteşe A, Örem A, Sönmez M, Aliyazıcıoğlu Y. *Rosa canina* extract has antiproliferative and proapoptotic effects on human lung and prostate cancer cells. Nutr Cancer. 2020;72(2):273–282.
5. Berkovich L, Earon G, Ron I, Rimmon A, Vexler A, Lev-Ari S. *Moringa oleifera* aqueous leaf extract down-regulates nuclear factor-kappaB and increases cytotoxic effect of chemotherapy in pancreatic cancer cells. BMC Complement Altern Med. 2013;13:212.
6. Hage-Sleiman R, Mroueh M, Daher CF. Pharmacological evaluation of aqueous extract of *Althaea officinalis* flower grown in Lebanon. Pharm Biol. 2011;49(3):327–333.
7. Al-Snafi AE. The pharmaceutical importance of *Althaea officinalis* and *Althaea rosea*: a review. Int J PharmTech Res. 2013;5(3):1378–1385.

8. Zhang Y, Kong F, Zhang L, Li C. Modulatory effect of *Althaea officinalis* L. root extract on cisplatin-induced cytotoxicity and cell proliferation in A549 human lung cancer cell line. Trop J Pharm Res. 2017;15(12):2647–2652.
9. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 2001;25(4):402–408.
10. Elmastaş M, Öztürk L, Erenler R, Gökçe İ. Determination of antioxidant activity of marshmallow flower (*Althaea officinalis* L.). Anal Lett. 2004;37(9):1859–1869.
11. Elmastaş M, Erenler R, Demirtaş İ, Öztürk L. Superoxide anion scavenging activity of marshmallow flower (*Althaea officinalis* L.). 7th Int Electron Conf Synth Org Chem (ECSOC-7). 2003;1–30.
12. Kobayashi T, Nakata T, Kuzumaki T. Effect of flavonoids on cell cycle progression in prostate cancer cells. Cancer Lett. 2002;176(1):17–23.
13. Shah SMA, Naveed A, Akram M, Shah PA. Pharmacological activity of *Althaea officinalis* L. J Med Plants Res. 2011;5(24):5662–5666.
14. Sadighara P, Gharibi S, Moghadam Jafari A, Jahed Khaniki G, Salari S. The antioxidant and flavonoids contents of *Althaea officinalis* L. flowers based on their color. Avicenna J Phytomed. 2012;2(3):113–117.
15. Ciobanu M, Pirvu L, Paun G, et al. Development of a new (bio)hybrid matrix based on *Althaea officinalis* and *Betonica officinalis* extracts loaded into mesoporous silica nanoparticles for bioactive compounds with therapeutic applications. J Drug Deliv Sci Technol. 2019;51:605–613.
16. Kadhum HH, Abd AH, Al-Shammari AM. Anti-proliferative activity of *Althaea officinalis* extracts on Iraqi breast cancer cell line AMJ13. Iraqi J Med Sci. 2021;19(2):--.
17. Farhat C, Younes H, Alyamani OA, Mrad M, Hourani N, et al. Chemical characterization and in vitro biological evaluation of aqueous extract of *Althaea officinalis* L. flower grown in Lebanon. J Herbal Med. 2022;34:100575.
18. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer. 1972;26(4):239–257.
19. Li X, Marani M, Yu J, Nan B, Roth JA, Kagawa S, Fang B, Denner L, Marcelli M. Adenovirus-mediated Bax overexpression for the induction of therapeutic apoptosis in prostate cancer. Cancer Res. 2001;61(1):186–191.
20. Chaabane W, User SD, El-Gazzah M, Jaksik R, Sajjadi E, Rzeszowska-Wolny J, Los MJ. Autophagy, apoptosis, mitoptosis and necrosis: interdependence between those pathways and effects on cancer. Arch Immunol Ther Exp (Warsz). 2013;61(1):43–58.
21. Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol. 2007;35(4):495–516.
22. Koff JL, Ramachandiran S, Bernal-Mizrachi L. A time to kill: targeting apoptosis in cancer. Int J Mol Sci. 2015;16(2):2942–2955.
23. Peña-Blanco A, García-Sáez AJ. Bax, Bak and beyond – mitochondrial performance in apoptosis. FEBS J. 2018;285(3):416–431.
24. Dozmorov MG, Hurst RE, Culkin DJ, Kropp BP, Frank MB, Osban J, Penning TM, Lin HK. Unique patterns of molecular profiling between human prostate cancer LNCaP and PC3 cells. Prostate. 2009;69(10):1077–1090.
25. Ravenna L, Principessa L, Verdina A, Salvatori L, Russo MA, Petrangeli E. Distinct phenotypes of human prostate cancer cells associate with different adaptation to hypoxia and pro-inflammatory gene expression. PLoS One. 2014;9(5):e96250.
26. Tai S, Sun Y, Squires JM, Zhang H, Oh WK, Liang CZ, Huang J. PC3 is a cell line characteristic of prostatic small cell carcinoma. Prostate. 2011;71(15):1668–1679.
27. Calabrese EJ, Mattson MP. How does hormesis impact biology, toxicology, and medicine? NPJ Aging Mech Dis. 2017;3:13.
28. Trachootham D, Alexandre J, Huang P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach. Nat Rev Drug Discov. 2009;8(7):579–591.
29. Son Y, Cheong YK, Kim NH, Chung HT, Kang DG, Pae HO. Mitogen-activated protein kinases and reactive oxygen species: How can ROS activate MAPK pathways? J Signal Transduct. 2011;2011:792639.
30. Rawlings JS, Rosler KM, Harrison DA. The JAK/STAT signaling pathway. J Cell Sci. 2004;117(Pt 8):1281–1283.
31. Hanahan D. Hallmarks of cancer: a 2012 perspective. Ann Oncol. 2012;23(9):ix23.
32. Weng CJ, Yen GC. Flavonoids, a ubiquitous dietary phenolic subclass, exert extensive in vitro anti-invasive and in vivo anti-metastatic activities. Cancer Metastasis Rev. 2012;31(1–2):323–351.
33. Eroglu C, Avci E, Secme M, Dodurga Y, Vural H, Kurar E. The combination effect of ferulic acid and gemcitabine on expression of genes related apoptosis and metastasis in PC3 prostate cancer cells. Eur J Biol. 2018;77(1):32–37.
34. Subhawa S, Naiki-Ito A, Kato H, Naiki T, Komura M, Nagano-Matsuo A, Yeewa R, Inaguma S, Chewonarin T, Banjerdpongchai R, Takahashi S. Suppressive Effect and Molecular Mechanism of *Houttuynia cordata* Thunb. Extract against Prostate Carcinogenesis and Castration-Resistant Prostate Cancer. Cancers (Basel). 2021;13(14):3403.
35. Satari A, Amini SA, Raeisi E, Lemoigne Y, Heidarian E. Synergetic Impact of Combined 5-Fluorouracil and Rutin on Apoptosis in PC3 Cancer Cells through the Modulation of P53 Gene Expression. Adv Pharm Bull. 2019;9(3):462-469.
36. Han R, Yang H, Li Y, Ling C, Lu L. Valeric acid acts as a novel HDAC3 inhibitor against prostate cancer. Med Oncol. 2022;39(12):213.
37. Tseng JC, Wang BJ, Wang YP, Kuo YY, Chen JK, Hour TC, Kuo LK, Hsiao PJ, Yeh CC, Kao CL, Shih LJ, Chuu CP. Caffeic acid phenethyl ester suppresses EGFR/FAK/Akt signaling, migration, and tumor growth of prostate cancer cells. Phytomedicine. 2023;116:154860.
38. Senthilkumar K, Arunkumar R, Elumalai P, Sharmila G, Gunadharini DN, et al. Quercetin inhibits invasion, migration and signalling molecules involved in cell survival and proliferation of prostate cancer cell line (PC3). Cell Biochem Funct. 2011;29(2):87–95.