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Research Article | Araştırma Makalesi

EARLY AND LATE STAGE DEPENDENT ANTICANCER EFFECTS OF *Laurus nobilis* SEED EXTRACTS ON PC3 PROSTATE CANCER CELLS

Laurus nobilis TOHUM EKSTRELERİNİN PC3 PROSTAT KANSERİ HÜCRELERİ ÜZERİNDEKİ ERKEN VE GEÇ EVRE BAĞIMLI ANTİKANSER ETKİLERİ

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ABSTRACT

Objective: This study explored the anticancer properties of *Laurus nobilis* seed extracts at different maturity stages on PC3 prostate cancer cells, aiming to understand how developmental stages influence biological activities.

Methods: Extracts from early-stage (green) and late-stage (black) *L. nobilis* seeds were tested on PC3 cells. Cytotoxicity, cell migration, wound healing, and gene expression were analyzed. HUVEC cells were used to assess toxicity on normal cells.

Results: Early-stage extracts showed low cytotoxicity and promoted cell migration and wound healing. Late-stage extracts exhibited potent dose-dependent cytotoxicity and significantly inhibited cell migration. Both extracts demonstrated lower toxicity towards HUVEC cells. Gene expression analysis revealed suppression of epithelial-mesenchymal transition and migration in cells treated with late-stage extracts.

Conclusion: The study highlights the stage-dependent anticancer potential of *L. nobilis* seed extracts. Late-stage extracts showed promising anticancer effects, while early-stage extracts promoted cell growth. These findings emphasize the importance of harvest time and maturity stage in determining the biological activity of *L. nobilis* extracts, providing valuable insights for future anticancer research and applications.

Keywords: *Laurus nobilis*, prostate cancer, seed extracts, stage-dependent effects, anticancer activity

ÖZ

Amaç: Bu çalışma, gelişim aşamalarının biyolojik aktiviteleri nasıl etkilediğini anlamak amacıyla, farklı olgunluk aşamalarındaki *Laurus nobilis* tohum özütlerinin PC3 prostat kanseri hücreleri üzerindeki antikanser özelliklerini araştırmıştır

Yöntem: Erken aşama (yeşil) ve geç aşama (siyah) *L. nobilis* tohumlarından elde edilen özütler PC3 hücreleri üzerinde test edilmiştir. Sitotoksisite, hücre göçü, yara iyileşmesi ve gen ekspresyonu analiz edilmiştir. Normal hücreler üzerindeki toksisiteyi değerlendirmek için HUVEC hücreleri kullanılmıştır

Bulgular: Erken aşama özütleri düşük sitotoksisite göstermiş ve hücre göçünü ve yara iyileşmesini teşvik etmiştir. Geç aşama özütleri, güçlü doz bağımlı sitotoksisite göstermiş ve hücre göçünü önemli ölçüde inhibe etmiştir. Her iki özüt de HUVEC hücrelerine karşı daha düşük toksisite göstermiştir. Gen ekspresyonu analizi, geç aşama özütleri ile tedavi edilen hücrelerde epitelyal-mezenkimal geçişin ve göçün baskılanmasını ortaya koymuştur.

Sonuç: Çalışma, *L. nobilis* tohum özütlerinin aşama bağımlı antikanser potansiyelini vurgulamaktadır. Geç aşama özütleri umut verici antikanser etkileri gösterirken, erken aşama özütleri hücre büyümesini teşvik etti. Bu bulgular, *L. nobilis* özütlerinin biyolojik aktivitesinin belirlenmesinde hasat zamanı ve olgunluk aşamasının önemini vurgulamakta ve gelecekteki antikanser araştırmaları ve uygulamaları için değerli bilgiler sağlamaktadır.

Anahtar Kelimeler: *Laurus nobilis*, prostat kanseri, tohum özleri, aşamaya bağlı etkiler, antikanser aktivite

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Introduction

Natural products cannot be easily obtained through chemical synthesis because of their complex structures. In recent years, new studies have been conducted on the use of various medicinal plants for cancer treatment. Medicinal plants have long been used in cancer treatment, and more than 3,000 plants used for this purpose have been listed in the literature. Recent research has revealed that Laurus nobilis may have therapeutic effects in some types of cancer.

Laurus nobilis L. (bay laurel), belonging to the Lauraceae family, is used as a folk remedy in many countries for the treatment of various diseases.[1] The essential oil obtained from the dried leaves of this plant, which is widespread in the Mediterranean region, is used in kitchens as a spice and flavoring agent.[2] *Laurus nobilis* L. (bay laurel) is a plant native to the Mediterranean region with aromatic leaves that has been used for centuries in cooking and folk medicine.[3] Traditionally, it has been prepared as an infusion or decoction and used to alleviate stomach and gas problems. Bay leaf oil is also used in the food industry as a flavoring agent and additive in animal feed.[4] It has been traditionally used to treat digestive system disorders, indigestion, and bloating. In addition, it has been reported that the bay laurel plant contains active components with antibacterial, antifungal, and antitumor properties.

Compounds isolated from plants are also noteworthy. Compounds such as Costunolide (CTL) and Lauroside B have been reported to possess antiproliferative potential in various cancer cell lines, while AETO has been reported to exhibit neuroprotective effects against dopamine-induced apoptosis and α-synuclein aggregation.[5] Costunolide (CTL), an active component of plants, has been shown to induce apoptosis in breast cancer cells (SK-BR-3).[5] Laurel leaf products have been shown to inhibit cell growth in human colon cancer cell lines (SW-480, Caco-2, HT-29, and HCT-116).[6] Furthermore, the chloroform extract of bay seeds has been reported to be effective against multidrug-resistant tumor cells expressing P-glycoprotein.[7] In contrast, ZnO and Ag nanoparticles have been produced using green synthesis methods with bay laurel extracts, and these nanoparticles have been shown to have anticancer and antibacterial effects.[8,9] Molecular docking studies have shown that 1,8-cineole, the dominant component in laurel essential oil, can form strong hydrophobic interactions in the binding sites of biological target proteins. These findings demonstrate that *L. nobilis* is a valuable natural resource in the food and traditional medicine sectors, as well as in pharmacology, biotechnology, and nanotechnology.

Our primary objective in this study was to investigate the anticancer effects of laurel plant seeds at early and late stages. To this end, methanol extraction was performed on seeds obtained at early and late stages, and the resulting extract was first tested on PC3 cells, a prostate cancer cell line. At the same time, it was also tested on healthy human umbilical vein endothelial cell (HUVEC). Based on the results obtained, a wound-healing study was conducted to investigate the wound-healing abilities of the materials. To further interpret the results of these experiments, an *in silico* analysis was performed for 1,8 cineol, the most abundant active ingredient, based on studies in the literature. This study revealed the effects of seed methanol extracts obtained at early and late stages on prostate cancer cells.

Methods

Plant Material and Preparation of Extracts

Different types of seeds (*Laurus nobilis* L., *Lauraceae*) were obtained from Altinoluk / Balikesir. The first group of seeds was classified as early harvest, and the second group as mature (late) harvest. The laurel plant was weighed before extraction and crushed in a mortar with liquid nitrogen. It was pulverized in a mortar and placed in 40mL of methanol / 10mL of water. Crushed laurels were added and centrifuged for 10 min, and this process was repeated thrice. The extract was filtered using Whatman No. 1 filter paper. It was dissolved in DMSO for the experiments.

Cell Culture

The prostate cancer cell line (PC3) and normal human umbilical vein cells (HUVEC) were purchased from the American Type Culture Collection (ATCC). PC3 and HUVEC cell lines were grown in DMEM (Sigma) containing L-glutamine, 10% fetal calf serum (Invitrogen), and penicillin-streptomycin (10,000 U/mL) at 37°C in an incubator with 5% CO_2 (v/v).[10,11]

Cytotoxicity Assay

To determine the antiproliferative effect of *Lauris nobilis* methanol extracts on the growth of PC3 and HUVEC cells, the MTT assay (Invitrogen, Australia) was used. The cancer cells were exposed to varying concentrations of purified methanol extracts and incubated for 24 h. Subsequently, 10 µL of MTT stock solution (1 mg/ml) was added to each well, and the plate was incubated for another 4 h. The plate was then drained, and the formazan product was dissolved in 100 µL of isopropanol/HCl solution. Finally, the absorbance at 550 nm was measured using a spectrophotometer (Multiskan MS; Thermo Fisher Scientific).[12,13]

Wound Healing Assay

The wound-healing assay began by seeding cells in 6-well plates and growing them to 90-95% confluence. A scratch wound was then created across the cell monolayer using a sterile 200 µL pipette tip, followed by gentle washing with PBS to remove detached cells. Fresh media containing treatments or vehicle controls were added, and images of the wound area were captured at 0 h using an inverted microscope. The plates were incubated at 37°C and 5% CO2, and additional images were taken of the same wound areas at 24 and 48h post-scratch.

Wound closure was quantified by measuring the wound area at each time point using image analysis software, and the percentage wound closure relative to 0 h was calculated for each condition. The treatment groups were compared using statistical analysis.

Real Time PCR Analysis

Studies were conducted to determine the gene expression levels in PC3 prostate cancer cells treated with methanol extract of *Laurus nobilis* buds. PC3 cells were cultured in 25 cm^2 flasks and treated with methanol extracts obtained from the early (green) and late (dark) developmental stages of *Laurus nobilis* buds. After 24 and 48 h of incubation, the cells were harvested by trypsinization. The collected cell suspensions were centrifuged at 4°C, and the pellets were thawed on ice prior to isolating RNA. Total RNA was extracted from both the control and treatment groups using the GeneJET RNA Purification Kit (Thermo Scientific, USA) following the manufacturer's instructions. The purity and concentration of the isolated RNA were determined spectrophotometrically at 260/280 nm using a Thermo Multiskan GO µDrop Plate Reader. Complementary DNA (cDNA) was synthesized from the isolated RNA samples, and gene expression levels were quantified using real-time quantitative PCR (qRT-PCR). For real-time PCR reactions, 5 µL of SYBR® Green PCR Master Mix, 1 µL of cDNA, and 0.5 µL (100 ng/µL) of each forward and reverse primer were used in a 10 µL reaction volume, as previously described.[12,14–16] Each reaction was performed in triplicate, and relative gene expression levels were calculated using the Livak ($2^{-}\Delta\Delta Ct$) method.

***In Silico* Analysis**

In this study, an *in silico* approach was used to assess the pharmacokinetic properties, drug-likeness, and toxicity profiles of the bioactive compounds present in *Laurus nobilis*. Initially, the phytochemical constituents of *Lauris nobilis* were identified through a literature review.[17] The chemical structures of the identified compounds were retrieved from the PubChem database and exported in the Simplified Molecular Input Line Entry System (SMILES) format for computational analysis. SwissADME (version 1.0) (http://www.swissadme.ch/index.php) was used to evaluate the physicochemical properties, gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability, and P-glycoprotein (P-gp) substrate. The admetSAR 2.0 (https://lmmd.ecust.edu.cn/admetsar1) database was used to predict human intestinal absorption (HIA), Ames mutagenicity, hepatotoxicity, and carcinogenic potential. PASS Online (https://www.way2drug.com/passonline/) was used to estimate the potential biological activity spectra of the compounds based on structure-activity relationships. All results were compiled and comparatively analyzed to identify the major compounds of *Lauris nobilis* with promising pharmacological potential.

Statistical Analysis

All experiments were performed in triplicate, and the data are presented as mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism software (version 9.0). For the cytotoxicity assay, dose-response curves were generated, and IC50 values (concentration causing 50% inhibition of cell viability) were calculated using nonlinear regression analysis. Differences in cell viability between the treatment groups were analyzed using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. The wound healing assay results were quantified by measuring the wound area at 0, 24, and 48 h post-scratch using ImageJ software. Wound closure rates were compared between the treatment groups using two-way ANOVA with Bonferroni correction for multiple time points. Statistical significance was set at $p<0.05$. Graphs were generated using GraphPad Prism to visually represent the data and the statistical outcomes.

Results

The effect of early and late period laurel methanol extract on cell viability

The cytotoxic effects of *Laurus nobilis* bud extracts on PC3 prostate cancer cells were evaluated using the MTT assay at incubation times of 24, 48, and 72 h. Cell viability was highly preserved at all concentrations (above 80%) in green (EARLY) bud extract applications, with only a slight decrease observed at the highest doses (500–1000 µg/mL) (Figure 1 a–c). This finding indicates that the extract in the early developmental stage did not cause significant toxicity in PC3 cells.

In contrast, the black (LATE) bud extract exhibited a pronounced, dose-dependent cytotoxicity profile (Figure 1 d–f). The IC_{50} values at 24, 48, and 72 h of incubation were determined to be 58.3, 63.7, and 85 µg/mL, respectively. At low concentrations (≤62.5 µg/mL), cell viability fell below 50%, whereas at concentrations of 250 µg/mL and above, it fell below 20%. Over time, a slight increase in the IC_{50} value at 72 h indicated partial adaptation or resistance development in the cell population. These results indicate that secondary metabolites (e.g., oxidized phenolics and terpenoids) that accumulate in the mature buds of *L. nobilis* exert potent antiproliferative and cytotoxic effects on PC3 cells. In contrast, the low toxicity of the green bud extract suggests that antioxidant compounds predominate in the early stages.

MTT assays were conducted on normal human endothelial cell lines (HUVEC) to determine the effect of *L. nobilis* bud extracts on cell viability. Cell viability was generally maintained within the range of 70–90% during 24-, 48-, and 72-hour incubations with green (EARLY) bud extracts (Figure 2 a–c). No significant cytotoxicity was observed at low concentrations (62.5–250 µg/mL), and

only a slight decrease in cell viability was detected at high doses (500–1000 µg/mL). This indicates that the early-stage extract has a good biocompatibility profile in HUVEC.

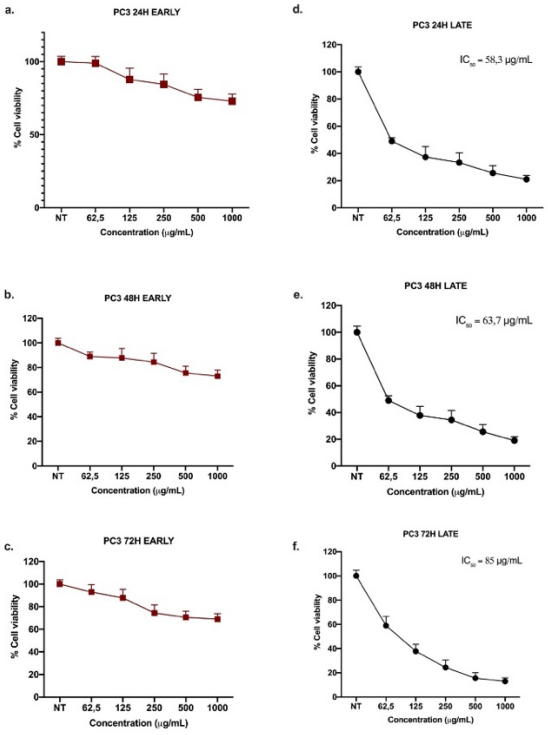

Figure 1. Cytotoxic effects of *Laurus nobilis* bud extracts on PC3 prostate cancer cells depending on time and maturity stage. (a–c) Cell viability percentages after 24, 48, and 72 h of treatment with green (EARLY) bud extract. No significant decrease in cell viability was observed with early-stage extract treatment; slight decreases were detected at high doses (500–1000 µg/mL). (d–f) Black (LATE) bud extract applications at the same time points showed marked dose-dependent cytotoxicity. The IC_{50} values were calculated as 58.3, 63.7, and 85 µg/mL for 24, 48, and 72-hour incubations, respectively. Data are expressed as the percentage of cell viability relative to that of the control group (NT, untreated cells) (mean ± SD, n=3).

In late (LATE) bud extract applications, a slight dose-dependent decrease in cell viability was observed (Figure 2 d–f). Viability rates remained at 60–80% over the 24–72 h interval, and no significant IC_{50} value was calculated. These results indicate that mature bud extracts have lower toxicity in normal endothelial cells than in cancer cells. Therefore, it was concluded that *L. nobilis* extracts have the potential to induce selective cytotoxicity in tumor cells while exhibiting higher dose tolerance in HUVEC cells.

The relationship between laurel's late-early extracts and wound healing ability

The effects of *L. nobilis* bud extracts on migration and epithelial-mesenchymal transition (EMT)-related gene expression in PC3 prostate cancer cells were evaluated using wound healing and qRT-PCR analyses.

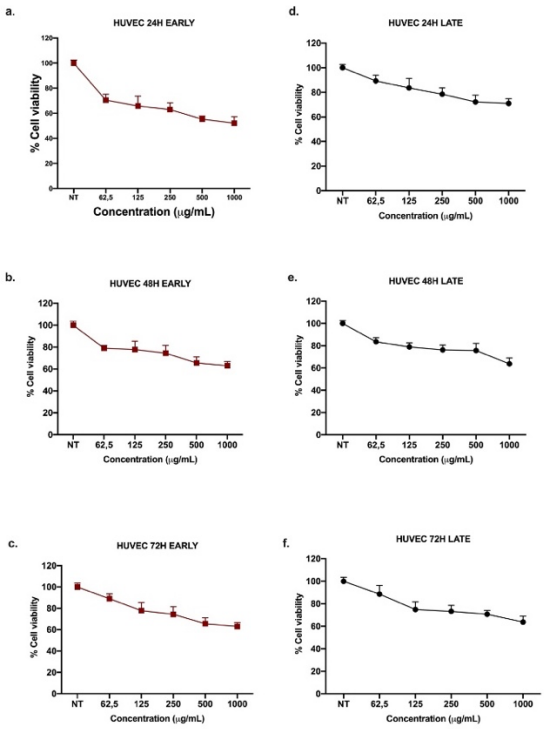

Figure 2. Time- and maturity-dependent cytotoxic effects of *Laurus nobilis* bud extracts on HUVEC endothelial cells. (a–c) Cell viability percentages after 24, 48, and 72 h of incubation with green (EARLY) bud extract. In the early-stage extract applications, no significant decrease in cell viability was observed at low and medium doses (≤250 µg/mL), whereas mild toxic effects were observed at high doses (500–1000 µg/mL). (d–f) In applications with black (LATE) bud extract at the same time points, a slight dose-dependent decrease was also observed, but overall, HUVEC viability was maintained above 60%. Data are shown as percentage of cell viability relative to the control group (NT, untreated cells) (mean ± SD, n = 3).

The effect of *L. nobilis* bud extracts on wound healing in PC3 cells was evaluated at 0, 6, and 24 h (Figure 3a-b). In the control group, the wound margin partially narrowed, showing approximately 40–50% closure after 24 h. In the group treated with (EARLY) bud extract, cells migrated intensively to the wound edge, and the wound area was largely closed at 24 h. In contrast, in the group treated with late (LATE) bud extract, cell migration was significantly reduced, the wound width was maintained, and the closure rate was significantly lower than that in the control group.

These results demonstrate that *L. nobilis* buds exhibit opposing biological effects depending on their maturity stage. While green bud extract is likely rich in antioxidants and phenolic compounds that support migration, mature bud extract contains high levels of oxidized metabolites, which suppress cell motility. The findings indicate that the green extract exhibits regenerative and wound-healing properties, whereas the black extract displays anti-migratory and potential anti-cancer properties.

While the wound line narrowed by approximately 50% at 24 h in the control group, the wound area was almost completely closed in cells treated with bud extract collected in the early stage (EARLY). In contrast, in cells

treated with bud extract collected in the late stage (LATE), the width of the wound line was largely preserved, indicating a significant suppression of cell migration. In the quantification analysis performed using ImageJ software, the migration rate exceeded 90% in the EARLY group at 24 h, whereas it fell below 40% in the LATE group. This finding demonstrates that *L. nobilis* buds exhibit opposite effects depending on their stage of maturity, with the early-stage extract increasing cell motility and the mature-stage extract producing an antimigratory effect (Figure 3a-b).

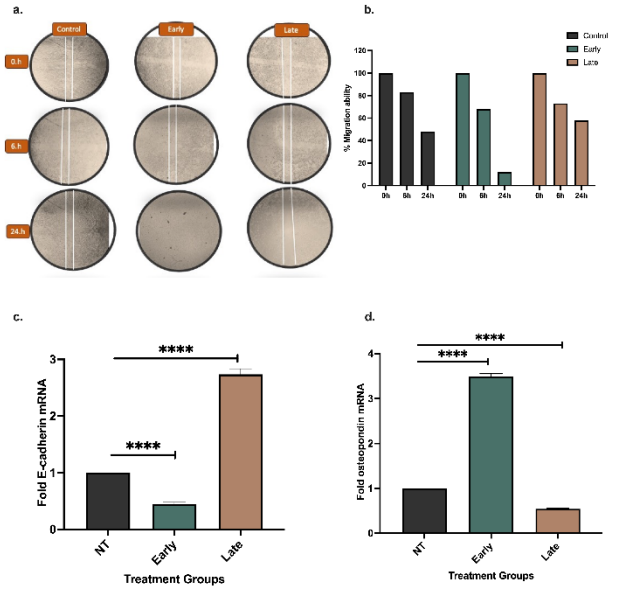

Figure 3. (a) Wound healing images of PC3 cells treated with control, early (EARLY, green bud), and late (LATE, black bud) extracts at 0, 6, and 24 h. (b) Wound area change was quantitatively analyzed using ImageJ software, and the relative migration rate (%) was calculated relative to the 0-hour value for each group. The wound area was almost completely closed at 24 h in cells treated with early bud extract, whereas the wound line width was maintained in cells treated with late bud extract. (c–d) mRNA levels of genes associated with epithelial-mesenchymal transition (EMT) were analyzed using qRT-PCR in the same groups. (c) E-cadherin (CDH1) gene expression was significantly increased in the LATE group ($p<0.0001$) and markedly decreased in the EARLY group. (d) Osteopontin (SPP1) expression was highly induced in the EARLY group and suppressed in the LATE group ($p<0.0001$).

According to the qRT-PCR results, the E-cadherin (CDH1) mRNA level increased significantly in the LATE group (approximately 2.8-fold, $p<0.0001$), whereas it decreased below the control level in the EARLY group. This suggests that the mature extract maintains the epithelial phenotype by enhancing cell-cell adhesion.In contrast, osteopontin (SPP1) expression increased approximately 3.5-fold in the EARLY group compared to the control group, whereas it decreased significantly in the LATE group ($p<0.0001$). The increase in osteopontin levels suggests that the early extract supports ECM remodeling and migration, while the late extract suppresses this process (Figure 3c-d).

Overall, these results indicate that *L. nobilis* buds regulate cell behavior differently depending on their developmental stage. The green (EARLY) bud extract induced an EMT-like transient migration response, consistent with E-cadherin suppression and increased osteopontin levels, while the black (LATE) bud extract exhibited an anti-migratory and potentially anti-cancer effect, characterized by increased E-cadherin and decreased osteopontin levels.

General Biological Activities of 1,8 Cineol, the Most Important Active Ingredient in *L.Nobilis*

The pharmacokinetic and toxicological properties of the methanol extract of *L. nobilis* L. were evaluated using *in silico* ADMET analyses. The data obtained (Table 1) show that the predominant phytochemicals in the extract exhibit favorable pharmacokinetic properties. Human intestinal absorption was 36.505%, indicating high gastrointestinal absorption potential. The compound was identified as a P-glycoprotein substrate but was determined not to be a P-gp I or II inhibitor, indicating a low risk of efflux inhibition with effective transport.

Table 1. *In silico* ADMET and toxicity analysis of 1,8 cineole, the active ingredient of the methanol extract of laurel (*Laurus nobilis* L.)

Activity	In silico analyses	1,8 cineole
Prediction of biological activity	P-glycoprotein Substrate	Yes
	P-glycoprotein I inhibitor	No
	P-glycoprotein II inhibitor	No
	Human Intestinal Absorption	96.505%
	CYP1A2 inhibitor	No
	CYP2D6 inhibitor	No
	CYP3A4 inhibitor	No
Prediction of toxicity	Mutagenic (AMES toxicity)	No
	Max. tolerated dose (human)	0.553 (log mg/kg/day)
	hERG I inhibitor	No
	hERG II inhibitor	Yes
	Oral Rat Acute Toxicity (LD50)	2,01(mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	2,029 (log mg/kg_bw/day)
	Hepatotoxicity	No
	Skin Sensitisation	Yes
	T. *Pyriformis* toxicity (IGC50, ug/L)	0,171 (log ug/L)
	Minnow toxicity	1.735 (log mM)
Pharmacokinetics	GI absorption	High
	BBB permeant	Yes
	P-gp substrate	No
	CYP1A2 inhibitor	No
	CYP2C19 inhibitor	No
	CYP2C9 inhibitor	No
	CYP2D6 inhibitor	No
	CYP3A4 inhibitor	No

According to cytochrome P450 (CYP) enzyme inhibition analysis, this compound does not inhibit CYP1A2, CYP2D6, CYP2C9, CYP2C19, or CYP3A4. This suggests a

low potential for drug-drug interactions. In toxicity analyses, the Ames test was negative (non-mutagenic), and hepatotoxicity and carcinogenicity were not detected. However, skin sensitization was predicted to be positive for all the compounds.

The maximum tolerated dose in humans was calculated as 0.553 log mg/kg/day, and the oral acute toxicity (LD_{50}) in rats was calculated as 2.01 mol/kg. Chronic exposure (LOAEL = 2.029 log mg/kg_bw/day) values also remained within the safe limits. The fish (IGC_{50} = 0.171 log µg/L) and minnow (1.735 log µmol/L) toxicity values indicated moderate environmental toxicity.

Discussion

Numerous cancer studies have been conducted on extracts obtained from *Laurus nobilis* L. (bay laurel), specific compounds, and nanoparticles derived from them. These studies have examined the potential cytotoxic, antiproliferative and apoptotic effects of these compounds on different types of cancer.

The volatile oil from fresh Palestinian bay leaves has been reported to exhibit a stronger antiproliferative effect than doxorubicin in MCF-7 cells.[7] However, it has also been reported that essential oil obtained from Algeria did not show a significant antiproliferative effect on MCF-7 and A549 cells.[18] The isolated compound costunolid (CTL) has been shown to induce potent cytotoxicity, particularly in SK-BR-3 cells, and this effect activates the mitochondrial apoptosis pathway via ROS production and lysosomal membrane permeability.[5] Furthermore, silver nanoparticles (AgNPs) synthesized using bay leaf extract inhibited the growth of MCF-7 cells.

Laurel extracts have been shown to inhibit cell growth and induce apoptosis in colon cancer cells. Fractions with different molecular weights trigger apoptosis in HT-29 cells through different ROS-mediated mechanisms.[19] Acetone extracts have been shown to exhibit cytotoxicity in HeLa cells.[20] Silver nanoparticle formulations similarly suppressed the growth of HeLa cells; however, peptide extracts did not show a significant antiproliferative effect.[8] Seed extracts have been shown to be sensitive to P-glycoprotein-expressing resistant CEM/ADR5000 cells.[7] Seskiterpenes isolated from laurel induce chromatin condensation and apoptosis in HL-60 cells. Furthermore, 1,8-cineole, the main component of laurel oil, induced apoptosis in HL-60 and Molt 4B cells.[21] The apolar fractions of bay laurel extracts induced cytotoxicity and apoptosis in neuroblastoma and glioma cells. In contrast, the AETO compound isolated from bay laurel exhibited a neuroprotective effect against dopamine-induced apoptosis.[22] ZnO nanoparticles synthesized using laurel extract have been shown to suppress A549 cell viability at high doses. However, no significant effects of the peptide extracts on A549 cells were observed.

Lauroside B inhibits the proliferation of different melanoma cells and suppresses NF-κB activation.[23] In addition, laurel fruit oil exhibited high cytotoxicity in C32 and ACHN cells. Cinnamtannin B-1 (CTB-1) inhibited proliferation in osteosarcoma cells, and this effect was reported to occur via the miR-1281/PPIF axis.[24] Laurel-based nanoparticle formulations, such as PLGA nanoparticles loaded with laurel essential oil, have been evaluated as controlled-release systems.[25] Furthermore, although not directly related to *L. nobilis*, gene therapy approaches (e.g., inducible Caspase 9 system) have been found promising in cancer treatment and have been considered in conjunction with laurel-centered research. Studies on the laurel plant and its components have indicated its anticancer potential in a wide variety of cancer models. This potential is particularly evident through apoptosis induction, proliferation suppression, and targeting of molecular pathways, such as ROS and NF-κB.

In this study, we compared the effects of extracts obtained from *Laurus nobilis* buds collected at different stages of maturity (green and black periods) on prostate cancer cells. Extracts collected during the green stage promoted cell migration and wound healing at 24 h, whereas extracts collected during the mature (black) stage significantly inhibited this process. This difference in effect is thought to stem from changes in the secondary metabolite composition during plant maturation.

The qualitative and quantitative profiles of phenolic, flavonoid, and terpenoid components change during the physiological maturation period of plants. *L. nobilis* is rich in flavonoids, phenolic acids, and monoterpenes (e.g., 1,8-cineole and α-terpineol) during the early developmental stage (green bud), and these compounds exhibit antioxidant and anti-inflammatory properties. These compounds can activate pathways associated with wound healing, such as NF-κB, ERK1/2, and MMP-2/9, by neutralizing intracellular reactive oxygen species (ROS).[26] This mechanism is consistent with the ability of green bud extract to increase cell migration in prostate cancer cells. During ripening, the oxidation and polymerization of phenolics in plant tissues increases, resulting in a decrease in antioxidant capacity. Simultaneously, the proportion of lipophilic compounds, such as oxidized terpenoids, tannins, and methyl eugenol, increases. These compounds can exhibit cytotoxic or antimigratory effects at high concentrations.[27] In an RNA sequencing-based study by Rodd et al., different molecular weight fractions of *L. nobilis* leaf extracts were found to activate distinct ROS-mediated apoptosis pathways in HT-29 colon cancer cells.[19] This finding indicates that changes in phytochemical composition are critical for determining the cellular fate.

Furthermore, the volatile and fixed oil compositions of *L. nobilis* showed significant differences depending on the harvest time and degree of ripeness. Awada et al. (2023) thoroughly examined the metabolite composition of *L. nobilis* leaves and fruits and reported that the amounts of major components such as β-ocimene, 1,8-cineole, and β-elemene varied according to ripening and geographical conditions.[17]

Viuda et al. (2007) demonstrated that metabolite diversity among Mediterranean aromatic plants,

particularly bay laurel, changes with maturity using metabolomic and chemometric analyses.[2] GC-MS and molecular docking analyses conducted by Jaradat et al. (2024) also revealed that essential oils obtained from fresh bay leaves possess anticancer activity and that 1,8-cineole is the key compound responsible for this effect. [28]

When the literature and current findings are evaluated together, it is understood that biological activity shifts with changes in compound composition throughout the development stage of *L. nobilis* buds. While antioxidant and regenerative effects are dominant in the green stage, oxidative stress and antiproliferative responses are dominant in the mature stage. This emphasizes that the harvest time and maturity stage of plant products are decisive parameters for biological activity. Therefore, it is believed that extracts obtained from *L. nobilis* at different maturity stages can be evaluated for different purposes in regenerative or anticancer applications.

The early stage (green) extract showed minimal cytotoxicity against PC3 cells, with cell viability remaining above 80% even at high concentrations. In contrast, the late stage (black) extract displayed potent dose-dependent cytotoxicity, with IC50 values ranging from 58-85 µg/mL over 24-72 hours of treatment. This suggests that the phytochemical composition of *L. nobilis* seeds changes significantly during maturation, with the accumulation of cytotoxic compounds in the later stages. Interestingly, both early and late-stage extracts exhibited lower toxicity towards normal HUVEC endothelial cells compared to PC3 cancer cells. The early extract maintained HUVEC viability above 70% at all concentrations tested, whereas the late extract caused a moderate decrease in viability, ranging from 60% to 80%. This differential effect on cancer versus normal cells is a desirable characteristic for potential anticancer agents, as it suggests some degree of selectivity.

The wound healing assay provided further insights into the biological effects of the extracts. The early-stage extract promoted rapid wound closure and cell migration in PC3 cells, with nearly complete wound healing observed at 24 hours. In contrast, the late stage extracts significantly inhibited cell migration and wound closure. Epithelial–mesenchymal transition (EMT) is a reversible program in which epithelial cells lose apico-basal polarity and cell–cell adhesion while acquiring motile, invasive features. A central molecular event is the down-regulation of E-cadherin (CDH1), the core adherens-junction protein that maintains epithelial integrity; reduced E-cadherin facilitates detachment and migration.[29] In contrast, osteopontin (SPP1) functions as a matricellular ligand that activates pro-migratory and pro-survival signaling (e.g., integrin/FAK, PI3K–AKT, NF-κB), thereby promoting EMT and cell motility. In our study, late-stage extract increased E-cadherin and decreased osteopontin, consistent with preservation of the epithelial phenotype and suppression of migration, whereas the early-stage extract showed the opposite trend, in line with the observed pro-migratory response.

The contrasting effects of early versus late stage extracts likely reflect changes in phytochemical composition during seed maturation. Early-stage seeds may contain higher levels of antioxidants and phenolic compounds that support cell growth and migration. As the seeds mature, there is likely an accumulation of cytotoxic secondary metabolites such as terpenoids that exhibit antiproliferative and anti-migratory effects. This is consistent with previous studies showing stage-dependent changes in metabolite profiles of *L. nobilis.* [17]

The *in silico* analysis of 1,8-cineole, a major component of *L. nobilis*, predicted favorable pharmacokinetic properties including high gastrointestinal absorption. The compound was not predicted to inhibit major cytochrome P450 enzymes, suggesting a low risk of drug interactions. Toxicity predictions were also largely favorable, with no mutagenicity or carcinogenicity detected. However, potential for skin sensitization was noted.

These findings expand our understanding of the anticancer potential of *L. nobilis* and highlight the importance of considering maturity stage when evaluating medicinal plants. The late-stage seed extract shows promise as a source of antiproliferative compounds against prostate cancer, while exhibiting lower toxicity to normal cells. Further research is warranted to identify the specific bioactive compounds responsible for these effects and elucidate their mechanisms of action. Additionally, in vivo studies will be crucial to assess the efficacy and safety of *L. nobilis* extracts for potential therapeutic applications in prostate cancer.

This study presents an in vitro assessment of the stage-dependent effects of *L. nobilis* seed extracts. Experiments were conducted with a prostate cancer line (PC3) and a normal endothelial line (HUVEC) using unfractionated crude methanolic extracts; therefore, further elucidation of the specific components responsible for the effects and generalizability to different cell types is required. EMT interpretation was based on mRNA-level measurements of a limited number of markers, while functional assessments were limited to viability and 2D migration. These issues do not diminish the observed results but point to the need for next steps to strengthen the clinical implications of the findings through biodirected fractionation, protein level/pathway validation, additional cell models, and in vivo studies.

In conclusion, this study investigated the anticancer effects of *Laurus nobilis* seed extracts at different maturity stages on PC3 prostate cancer cells. The results demonstrated distinct biological activities depending on the developmental stage of the buds. Early-stage (green) extracts exhibited low cytotoxicity and promoted cell migration and wound healing, while late-stage (black) extracts showed potent dose-dependent cytotoxicity and significantly inhibited cell migration. Both early and late-stage extracts showed lower toxicity towards normal HUVEC cells, suggesting a potential selective effect on cancer cells. Gene expression analysis supported these findings, indicating a suppression of epithelial-

mesenchymal transition (EMT) and migration in late-stage extracts. These results highlight the importance of harvest time and maturity stage in determining the biological activity of *L. nobilis* extracts and provide valuable insights into their stage-dependent anticancer potential.

Compliance with Ethical Standards

In this study, a prostate cancer cell line and plant extracts were used, and no experiments involving human subjects or animals were conducted. Therefore, no ethics committee approval was applicable or included.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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