

## Research Article | Araştırma Makalesi

# ANTICARCINOGENIC EFFECTS OF AVOCADO AND PEANUT ON HUMAN PROSTATE (PC3) AND LUNG CANCER (A549) CELLS

## AVOKADO VE YER FISTIĞİNİN İNSAN PROSTAT (PC3) VE AKCİĞER KANSERİ (A549) HÜCRELERİ ÜZERİNDEKİ ANTİKANSER ETKİLERİ

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### ABSTRACT

**Objective:** In recent years due to their natural ingredients phytotherapeutic sources have become increasingly needed as alternatives to chemotherapeutic drugs. Avocado and peanut were shown to contain diverse bioactive compounds exhibit anti-oxidant, anti-inflammatory and anti-proliferative effects. We aimed to determine and compare the anticarcinogenic effects of Bacon and Fuerte avocado, Osmaniyee peanuts on the inhibition the viability of human prostate carcinoma (PC3) and human lung carcinoma (A549) cells.

**Methods:** Three kilograms of avocados and peanuts were purchased domestically produced. Avocados and peanuts were divided into three parts. Avocado and peanut parts were dissolved in 96% and 60% ethanol. A549 and PC3 cells were incubated with 200µg/ml ethanol extracts of avocados and peanuts for 24 hours. PC3 and A549 cancer cell viability analysis was performed using CCK-8 Kit.

**Results:** All parts of Bacon and Fuerte avocado were cultured with A549 and PC3 cells caused decrease in the viability of both cancer cells. All parts of peanut caused decrease in viability of A549 but there was no difference for PC3. Also, the peel and seed parts in 96% ethanol of Bacon and Fuerte avocado caused more decrease than pulp part for A549 and PC3. The inner peel and kernel parts in 96% ethanol of peanut caused more decrease than outer peel part for A549 but there was no difference for PC3.

**Conclusion:** Peel and seed parts of avocados and outer sheel and kernel parts of peanuts can serve as valuable sources to reduce cancer cell viability.

**Keywords:** Avocado, peanut, human lung carcinoma, human prostate carcinoma

### Öz

**Amaç:** Son yıllarda, kemoterapi ilaçlarına alternatif olarak, doğal içerikleri nedeniyle bitkisel tedavi kaynaklarına duyulan ihtiyaç giderek artmıştır. Avokado ve yer fıstığı gibi gıdaların, anti-oksidan, anti-inflamatuar ve anti-proliferatif etkiler gösteren çeşitli biyoaktif bileşimler içerdiği gösterilmiştir. Bacon avokado, Fuerte avokado ve Osmaniyee fıstığının insan prostat kansinomu (PC3) ve insan akciğer kansinomu (A549) hücrelerinin canlılık kapasitesini inhibisyon etkilerini dolayısıyla antikanser etkilerini belirlemeyi ve karşılaştırmayı amaçladık.

**Yöntem:** Yerli üretim olan üç kilo avokado ile yer fıstığı satın alındı. Avokadolar ve yer fıstığı üç kısma ayrıldı. Daha sonra avokado ve yer fıstığının kısımları %96 ve %60 etanolde çözüldü. A549 ve PC3 hücreleri, 200 µg/ml konsantrasyonundaki avokado ve yer fıstığı özütlarının etanol çözeltilerile 24 saat inkübe edildi. PC3 ve A549 kanser hücrelerinin canlılık analizi, CCK-8 Kiti kullanılarak gerçekleştirildi.

**Bulgular:** Bacon ve Fuerte avokado'nun A549 ve PC3 hücreleriyle kültür edilen tüm kısımları her iki kanser hücresinin de canlılığında azalmaya neden oldu. Fıstığın tüm kısımları A549 hücrelerinin canlılığında azalmaya neden oldu, ancak PC3 hücrelerinde herhangi bir fark gözlenmedi. Ayrıca, A549 ve PC3 için Bacon ve Fuerte avokadonun %96'lık etanoldeki kabuk ve çekirdek kısımları, etli kısma kıyasla daha fazla azalmaya neden olmuştur. A549 için Osmaniyee fıstığının %96'lık etanoldeki iç kabuğu ve çekirdek kısmı dış kabuk kısmına kıyasla daha fazla azalmaya neden olurken, PC3 için bir fark gözlemlenmedi.

**Sonuç:** Avokadonun kabuk ve çekirdek kısımları ile yer fıstığının dış kabuk ve iç kısımlarının kanser hücrelerinin canlılığını azaltabileceği sonucuna varıldı.

**Anahtar Kelimeler:** Avokado, yer fıstığı, insan akciğer kansinomu, insan prostat kansinomu

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## Introduction

Cancer is one of the most important health problems, characterized by abnormal and uncontrolled cell proliferation and is one of the leading causes of mortality worldwide.<sup>1,2</sup> Although chemotherapeutic therapy are essential, they have severe side effects for normal and cancer cells. In recent years, due to their easy accessibility, low cost, and natural ingredients there has been an increasing need for phytotherapeutic sources as alternatives to chemotherapeutic drugs.<sup>3</sup> Plant-derived foods like avocado and peanut suggested that contain diverse bioactive compounds such as phytochemicals and phytophenols that may exhibit anti-oxidant, anti-inflammatory and anti-proliferative effects potentially preventing carcinogenesis.<sup>1,4-6</sup> Although many studies are preclinical and observational, the available evidence suggests mechanisms through which these plants might contribute to cancer prevention or regulation.

Avocado, which native to central Mexico is also cultivated in many countries, including Türkiye.<sup>7</sup> There are different types of avokados like Fuerte, Bacon, Hass, Reed and Zutano. Bacon avocados are oval and medium-sized, the average fruit weight is 250-300 grams. This species was discovered by James Bacon in 1954, is a "Type B" avocado that can with stand temperatures down to -5 degrees Celsius. Fuerte avocados are pear-shaped, medium to large sized and weighed between 140 and 400 grams. It is a climacteric fruit containing approximately 20 vitamins and minerals. The vitamins are B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B9 (folic acid), vitamin C (ascorbic acid), vitamin E, vitamin K, potassium, and magnesium. It also contains lutein, beta-carotene, omega-3 and omega-6 fatty acids. Due to its rich nutritional values avocado's production and various scientific researches are increasing day by day in our country and in the world.<sup>6-8,10</sup> As a result of these rich nutrients, minerals and fiber it has been found to regulate digestive system, contribute cardiovascular health by lowering cholesterol, protect eyes health and have antioxidant and antiproliferative effects against different cancer types like colorectal, lung, prostate and bladder. In different studies about avocados have been shown to inhibit the viability and proliferation of cancer cells.<sup>11,12</sup>

Peanut (*Arachis hypogaea*) of which homeland is South America is also produced in the Mediterranean region especially in Adana and Osmaniye in Türkiye.<sup>4</sup> It has rich bioactive compounds such polyphenols, resveratrol, procyanidins, flavonoids and vitamins (B1, B2, B3, B6, and B9) tryptophan, zinc and magnesium.<sup>13,14</sup> It's seeds contain 45-60% oil, 20-30% protein and 18-25% carbohydrates and can be pressed for oil production or roasted and served as a snack. Depending on these compounds, recent studies have suggested that peanuts inhibited the viability of various cancer types including breast, prostate, pancreatic, stomach and colorectal

cancers, head and neck squamous cell carcinomas, ovarian carcinoma, and hematological malignancies.<sup>15,16</sup> In this study, we aimed to determine and compare the inhibition of the viability capacity of Bacon avokado, Fuerte avocado (in peel, pulp and seed parts) and Osmaniye peanuts (in outer shell, inner shell and kernels parts) on human prostate carcinoma (PC3) and human lung carcinoma (A549) cells.

## Methods

### Extraction of Fuerte Bacon Avocados and Osmaniye Peanuts

Three kilograms of fuerte and bacon avocados and peanuts were purchased domestically produced. Avocados were taken from Alanya. Peanuts were supplied from Osmaniye. Avocados and peanuts were cut into pieces. Bacon and fuerte avocados were also divided into three parts: peel, pulp and seed parts were extracted separately (Figure 1). The peanuts were divided into three parts: outer peel, inner peel, and kernels were extracted separately (Figure 2). After grinding, avocado (~1 g) and peanut (~0.5 g) samples were weighed and both of them were dissolved in 10 ml 96% and 60% ethanol purity ethyl alcohol and liquefied by applying a certain amount of heat and pressure. Then, they were subjected to vortexing for 10s, ultrasonication (VWR, USA) for 15 min, and centrifugation (Hettich, Germany) at 4500 rpm/4°C for 10 min, and kept in a dark and shaded area. The extracts (30 mm x 120 mm, 50 mL, 2.5 cm diameter) were transferred to a sterile test tube and stored in a deep freezer at -20 degrees. The supernatants obtained after centrifugation were transferred to a separate tube and the same procedures were repeated once more. Finally, the two supernatants were combined, and the final volume was made up to 20 mL. The prepared extracts were stored at -20°C until spectrophotometric analysis.

### Cell Culture of A549 and PC3 Cell Lines

A549 (lung carcinoma CCL185, ATCC) and PC3 (prostate adenocarcinoma CRL 1435, ATCC) were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS), 1% (2 mM) L-glutamine (L-Gln) and 1% (100U/mL / 100µg/mL) penicillin/streptomycin (complete medium) in 25 cm<sup>2</sup> tissue culture flasks from Corning (Charlotte, USA) at 37°C in a humidified incubator containing 5% CO<sub>2</sub> pressure incubated for 24 h and placed in an incubator containing 5% CO<sub>2</sub> at 37°C (Sanyo, Osaka, Japan). Microscopic examination showed that the cells had attached to the flask the following day and multiplied in the subsequent days. When the cells reached 70-80% confluence, they were passaged according to the manufacturer's protocol (Cell Applications, San Diego, USA).

The cells were used in experiments when they reached passage 3. A549 and PC3 cells that were not cocultured

with avocado and peanut extracts were evaluated as control groups.

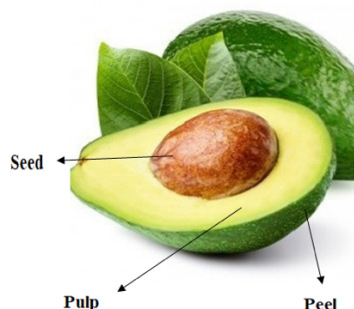


Figure 1. Peel, pulp and seed parts of an avocado

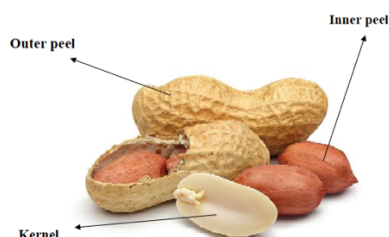


Figure 2. Outer peel, inner peel and kernel parts of a peanut

### Co-culture Experiments of A459 and PC3 with Avocado's and Peanut's Extracts

A549 and PC3 cells ( $3 \times 10^5$ /well in a 6-well plate) were incubated for 24 h before extracts were added. Then A549 and PC3 cells were incubated with 60% and 96% of 200  $\mu\text{g/ml}$  extracts of Bacon avocado, Fuerte avocado (peel, pulp and seed parts) and Osmaniye peanuts (outer shell, inner shell and kernels parts) for 24 hours. To enable an initial comparative screening across the large number of fractions, all extracts were first evaluated at a single fixed concentration (200  $\mu\text{g/ml}$ ), a commonly used approach in preliminary phytochemical cytotoxicity screening to prioritize active candidates for subsequent dose–response/IC50 analyses.<sup>17,20</sup>

### Cell Viability Analysis Using CCK-8 (Cell Counting Kit-8)

PC3 and A549 cancer cell viability analysis was performed using a CCK-8 Kit (Abbkine, Georgia, USA). Cell viability was assessed using a CCK-8 kit (Abbkine, USA). Briefly, after 24 h treatment in 6-well plates (as described above), cells were gently washed with PBS to remove residual extracts, detached using trypsin-EDTA, neutralized with complete medium, and counted using a hemocytometer/automated cell counter. Cells were then re-seeded into 96-well plates at  $1.5 \times 10^4$  cells/well in 100  $\mu\text{L}$  complete medium and incubated for 24 h to allow re-attachment. Subsequently, 10  $\mu\text{L}$  of CCK-8 reagent was added to each well and plates were incubated for 3 h at 37°C in 5%  $\text{CO}_2$ . Absorbance was measured at 450 nm using a microplate reader (BioTek Instruments, USA). Blank wells containing medium plus CCK-8 without cells were used for background subtraction. Viability was calculated relative to the corresponding control group.

Experiments were performed in three independent biological repeats, and each condition was measured in triplicate technical wells.

### Statistical Analysis

The results were presented as means  $\pm$  standard deviation (SD). Statistical analyses were conducted using GraphPad Prism 8 software (San Diego, USA). A one-way ANOVA was performed to compare the quantitative data across the groups. If the ANOVA results were significant, the Tukey's post-hoc Test was utilized to compare the means of the groups. Statistical significance for all analyses was set at a significance level of  $p < 0.05$ .

### Results

The peel, pulp and seed parts of Bacon and Fuerte avocados which were cultured with A549 and PC3 cancer cells, all parts of them were caused an important decrease of the viability of both cancer cells (Figure 3, Figure 4, Figure 5, Figure 6).

Also, the outer peel, inner peel and kernel parts of Osmaniye peanut, which was cultured with A549 and PC3 cells, all parts of it were caused a decrease in viability and proliferation of A549 but there was no difference for PC3 (Figure 7, Figure 8).

When the alcohol ratios of extracts were compared within themselves, 96% ethanol of the peel and seed parts of Bacon and Fuerte avocado were caused more decrease than pulp of both A549 and PC3 cancer cells (Figure 3, Figure 4, Figure 5, Figure 6).

96% ethanol of the inner peel, and kernel parts of Osmaniye peanut was caused more decrease than outer peel part of A549 but there was no difference for PC3 (Figure 7, Figure 8).

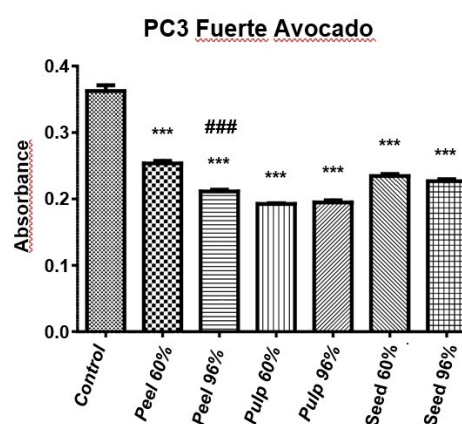
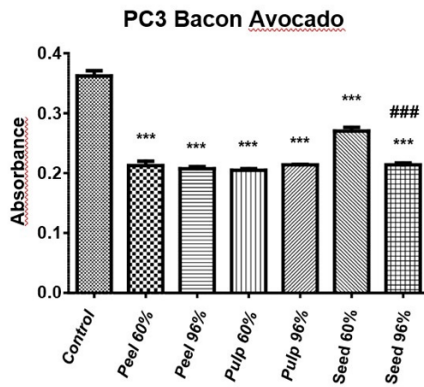
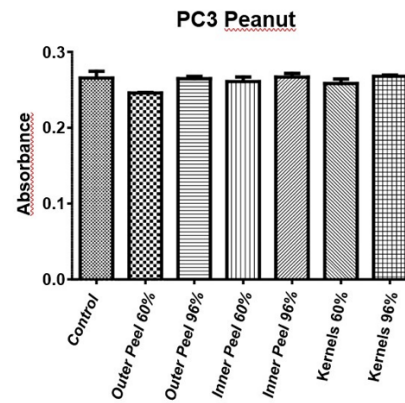


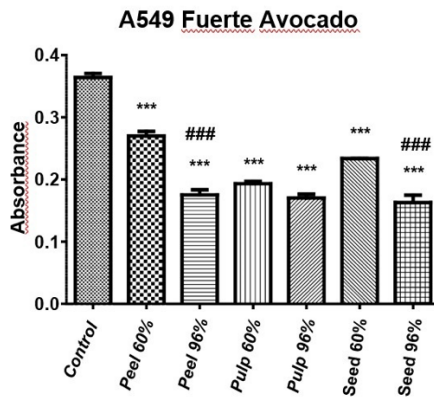
Figure 3. Compared to the control group (only PC3 cells), the preservation of PC3 cell viability in the extract parts of peel, pulp, and seed in Fuerte avocado found to be significantly reduced (\*\*\*  $p < 0.001$ ). When ethanolic extracts compared within themselves, the viability of PC3 cells cultured with 96% peel extract had more decrease compared to 60% in Fuerte avocado (###  $p < 0.001$ ).



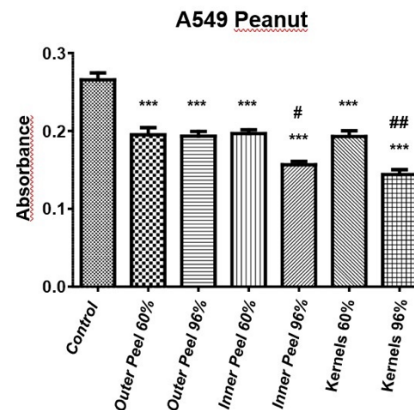
**Figure 4.** Compared to the control group (only PC3) the preservation of PC3 cell viability in the extract parts of peel, pulp, and seed extract parts in Bacon avocado found to be significantly reduced (\*\* $p < 0.001$ ). When ethanolic extracts were compared within themselves the viability of PC3 cells cultured with 96% seed extract had more decrease compared to 60% in Bacon avocado (###  $p < 0.001$ ).



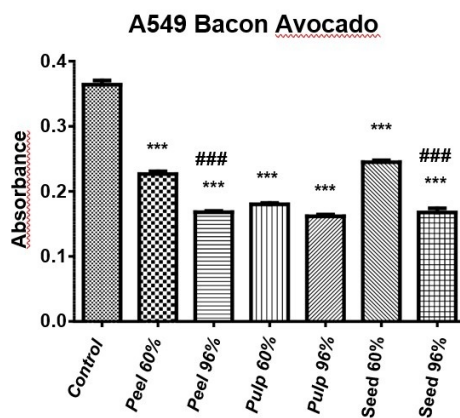
**Figure 7.** Compared to the control group (only PC3), the preservation of PC3 cell viability in the outer peel, inner peel and kernels extracts in Osmaniye peanut found no difference ( $p > 0.05$ ). When ethanolic extracts were compared within themselves, the viability of PC3 cells cultured with the outer peel, inner peel and kernels had no difference in Osmaniye peanut ( $p > 0.05$ ).



**Figure 5.** Compared to the control group (only A549), the preservation of A549 cell viability in the peel, pulp and seed extracts in Fuerte avocado found to be significantly reduced (\*\* $p < 0.001$ ). When ethanolic extracts were compared within themselves, the viability of A549 cells cultured with 96% seed and peel extracts had more decrease compared to 60% in Fuerte avocado (###  $p < 0.001$ ).



**Figure 8.** Compared to the control group (only A549), the preservation of A549 cell viability in the outer peel, inner peel and kernels extracts of peanut, was found to be significantly reduced (\*\* $p < 0.001$ ). When ethanolic extracts were compared within themselves, the viability of A549 cells cultured with 96% inner peel and kernels extracts had more decrease compared to 60% ethanol in Osmaniye peanut (###  $p < 0.001$ ).



**Figure 6.** Compared to the control group (only A549), the preservation of A549 cell viability in the peel, pulp and seed extracts in Bacon avocado found to be significantly reduced (\*\* $p < 0.001$ ). When ethanolic extracts were compared within themselves, the viability of A549 cells cultured with 96% seed and peel extracts had more decrease compared to 60% in Bacon avocado (###  $p < 0.001$ ).

## Discussion

Cancer is major public health problem, leading cause of mortality in the world after cardiovascular diseases. Studies suggest that the potential chemopreventive activity of avocado fruit especially peel and seed parts of avocado influence on cell cycle arrest, decrease viability and proliferation and promote apoptosis in cancer cell lines in vitro.<sup>11, 21</sup>

Also, avocado seed extracts with high polyphenol contents have been shown to reduce viability of human breast, lung, colon and prostate cancer cells.<sup>22, 23</sup> An acetone extract from avocado has been found to inhibit the viability of prostate cancer cells in vitro.<sup>21</sup> In a study four different avocado extracts were tested against esophageal squamous carcinoma and colon adenocarcinoma cells. All extracts significantly inhibited cancer cell viability.<sup>25</sup> In a different study avocado seed

extracts showed cytotoxicity and extending doubling time in MCF-7 breast cancer cells, indicating reducing viability and proliferation.<sup>26</sup> In a prospective study combining data from women and men, avocado consumptions (1b serving/week) were associated with reduced risk of total cancer and site-specific colorectal, lung, bladder cancers.<sup>27</sup>

Similar to these studies we detected the highest effects of peel and seed extracts of both Bacon and Fuerte avocados for reducing the viability of A549 cells. In addition, 96% of peel extracts of Fuerte avocado and 96% of seed extracts of Bacon avocado for reducing the viability of PC3 cells. As a result, we found that 96% extracts were more effective than 60% extracts. Peanuts (*Arachis hypogaea*) are rich in bioactive compounds such polyphenols, resveratrol, flavonoids and vitamins which have been studied for their potential anticancer properties. Recent studies have suggested that these compounds particularly those in peanut's outer shell and kernels parts reduced cancer cell viability and proliferation, in various cancer cell types.<sup>28-30</sup> In a study with DU145 prostate carcinoma cells showed that peanut kernels effectively reduced cell proliferation.<sup>29</sup> In another study peanut kernels against colorectal cancer lines suppressed cell viability and proliferation.<sup>31</sup>

On a dose-response basis, even small increases in daily purchases were related with reduced risk for several cancer types.<sup>32</sup> Resveratrol, a polyphenol found in peanuts kernels has shown in experimental studies to enhance natural killer (NK) cell activity, suggesting that a potential mechanism by which peanut compounds contribute to reduce the cancer cell's viability.<sup>33</sup>

Like these studies we also found the highest reducing effects of %96 inner shell and kernels extracts of peanuts in the viability of A549 cancer cells. A limitation of this study is that cytotoxicity was assessed at a single test concentration (200 µg/mL); therefore, dose-response relationships and IC50 values remain to be established in future studies.

According to all our results we suggest that peel and seed parts of avocados and outer shell and kernels parts of peanuts which are rich in polyphenols and flavonoids can serve as valuable sources of natural antioxidants and reduce cancer cell viability.

Foods like avocados and peanuts may be effective in preventing cancer risk and can be used as natural resources to support chemotherapy and other cancer treatments.

While various studies suggest that both avocados and peanuts may contribute to reducing cancer risks, further research and clinical trials into their anti-cancer mechanisms are needed to confirm.

#### Compliance with Ethical Standards

For this type of study formal ethical consent is not required.

#### Conflict of Interest

The authors disclose that they have no conflict of interest to declare.

#### Author Contribution

PC; Concept, PC, SD; Data collection and/or processing, PC; Analysis and/or Interpretation, PC, SD; Literature review, PC, SD; Writing the article.

#### Financial Disclosure

None of the authors of this manuscript had any financial relationships with other individuals or organizations.

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