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# Direct Impact of Pomegranate Juice and Peel Extracts on Prostate Cancer Progression - Inhibition of Proliferation, Migration, and Colony Formation in Prostate Cancer Cell Lines

*S. Philp*
*The University of Sydney, Australia*
*sphilp@sydney.edu.au*

***Abstract***

*Prostate cancer, which affects millions of men worldwide, is a prevalent form of cancer. The risk factors associated with it include age, genetics, and diet. Despite advances in cancer research, the quest for effective treatments and preventive measures against this disease remains an ongoing challenge. Pomegranate, a fruit known for its antioxidant-rich juice and bioactive peel extracts, has been identified as a prospective therapeutic intervention for various health ailments, including cancer. The current study looked at the direct effects of the juice of pomegranates and peel extract on the advancement of prostate cancer. Its ability to suppress the colony formation, motility, and proliferation of prostate cancer cell lines as cancer cells was assessed. The decline in cell survival after an injection of PP extract was measured using the Mean Transit Time (MTT) test to do this. Prostate cancer cells' ability to form colonies and to be inhibited from migrating was also evaluated using the clonogenic assay and wound healing experiment conducted with the help of pomegranate extracts. The results revealed promising inhibitory effects of pomegranate extracts on these key cancer hallmarks, suggesting their potential as complementary therapeutic agents for prostate cancer.*

***Keywords****: Natural extracts, Anti-cancer, Antioxidants, Pomegranate, Cytotoxicity*

## 1. Introduction

Globally, prostate cancer is the most prevalent noncutaneous cancer in males and is responsible for a considerable percentage of cancer-related fatalities [1][2]. Growing older is associated with a higher incidence and death rate of prostate cancer. The observed discrepancy has been postulated to arise from social, environmental, and genetic variations. The prevalence of prostate cancer, along with the slow progression of several tumors and the possibility of adverse treatment outcomes, has sparked debate over the effectiveness of screening and early diagnosis [3][4]. While early detection and treatment options, such as surgery, radiation therapy, and chemotherapy, have improved survival rates, these approaches often come with various side effects and limited efficacy during a later stage of the illness [5]. Consequently, interest is rising in exploring alternative and adjunctive therapies, especially those that harness the potential of natural compounds [6][7][8]. The field of ethnopharmacology has facilitated the exploration of traditional medical practices, leading to the identification of several therapeutic compounds. For instance, digitoxin is a cardiac

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glycoside extracted from Digitalis Purpurea (foxglove). This substance has demonstrated potential in managing cardiovascular disorders and has also been used in cancer treatment plans [4]. In 2020, a total of over 20 million individuals were diagnosed with cancer, leading to the unfortunate outcome of >8 million deaths. These deaths accounted for approximately 14% of the overall reported mortality rate. The prevalence of cancer cases is steadily rising as a result of rapid population expansion and increased life expectancy, with a projected surge of 40% by the year 2040 [1]. It is critical to find novel approaches to cancer therapy to lessen patient suffering and save costs related to today's expensive treatments. Toxicology to the body's healthy cells is one of the main drawbacks of chemotherapy, resulting in significant side effects like fatigue, alopecia, anemia, and increased susceptibility to bruising and bleeding, among other adverse consequences [9]. The pharmacological properties and broad structural diversity of plant-based substances and their byproducts have demonstrated much potential for developing chemotherapy drugs [10]. FDA is the United States Food and Drug Administration authorized the semi-synthetic derivative with a natural taxoid called cabacitaxel in 2010 as an alternate treatment for prostate cancer that is hormone-refractory.

Fruit and juice from the pomegranate plant (Punica Granatum) are frequently consumed. Pomegranate fruit extracts have been sold as dietary supplements lately due to their possible health advantages. For instance, by upregulating the expression for CYP7B1, the gene that encodes for the enzyme known as oxysterol seven alpha-hydrolase, it has been discovered that ingestion of pomegranate extract lowers liver and blood cholesterol levels. The documented cholesterol-lowering benefits of pomegranate extract are further enhanced by its downregulation of SREBP1, a gene that contributes to the regulation of sterol production. The enzyme CYP7B1 plays a crucial role in maintaining the balance of cholesterol, bile acid, and oxysterol levels, whereas SREBP1 governs the expression of genes involved in lipid and cholesterol synthesis [11].

Pomegranate is known for its unique combination of bioactive compounds, including polyphenols, flavonoids, and anthocyanins, primarily found in juice and peel [12][13]. These substances have been demonstrated to possess antioxidant, anti-cancer, and anti-inflammatory properties, making them an attractive candidate for cancer therapy and prevention [14][15]. Our goal in this research was to examine the direct impact of pomegranate juice and peel extracts on prostate cancer progression.

## 2. Materials and methods

Pomegranate juice and peel extraction: The ripe fruits of native pomegranate plants were harvested from the rural region to get unaltered, naturally occurring fruit. The peel was manually separated and air-dried at room temperature for 4 to 6 days. Before extraction, the dried peel was crushed using a laboratory mill. Subsequently, a powdered form of pomegranate peel weighing 50g was subjected to extraction using a 50% ethanol solution. The extraction process was carried out in an ultrasonic bath for 40 minutes, maintaining a temperature of 60ºC [16]. The extract was filtered and then evaporated in a rotating evaporator (Büchi R-210, Flawil, Switzerland) to ensure it was dry. The preparation of pomegranate juice involved sourcing pomegranate fruits using standardized procedures. In summary, the fruits were cleaned, followed by juice extraction using pressing and centrifugation techniques.

Cell culture: ALVA-41, NCI-H660, ARCaP (also known as MDA PCa 1), and LNCaP are human cancer cell lines employed in this work through the National Centre of Cell Science, Pune. The cells were cultured as monolayers in RPMI 1640 media (Sigma-Aldrich Chemie

GmbH, Steinheim, Germany), supplemented with 10 percent fetal human blood serum (Sigma-Aldrich Chemie GmbH) and 0.9 percent sodium chloride solutions with 5000U penicillin and 5 mg/mL streptomycin. As per the guidelines provided by the manufacturer (Heraeus, Hanau, Germany), the cells were cultured at 37°C in a controlled environment with a concentration of 5% CO2.

Cytotoxicity testing (MTT Assay): Observing the guidelines provided by the manufacturer (Germany's Sigma-Aldrich), this study performed the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) using the tetrazolium reduction test to assess a loss in cell viability after the injection of PP extract. After exposure to varying concentrations of extracts, the cells were incubated for 24, 48, and 72 hours. A microplate reader measured the absorbance at 570 nm in wavelength. According to Lopez-Lázaro [17], the degree of selectivity, sometimes called the selectivity index (SI), is the ratio of a sample's hazardous concentration to its effective bioactive concentration. Divided by the IC50 values obtained from the malignant cell line (A549) for every experiment, the mean of the IC50 readings for the standard cell line was determined.

Wound healing experiment: This was employed to assess the migratory inhibitory effects of pomegranate extracts. The cells were let to reach confluence, at which point a scratch was introduced, and the subsequent rate of wound closure was observed during a designated period.

Clonogenic Assay: Colony Formation Assay: The capacity of prostate cancer cells to generate colonies in the presence of pomegranate extracts was evaluated. The cells were collected using the trypsinization process using a 0.25% trypsin solution obtained from Serva Feinbiochemica in Heidelberg, Germany. The cells were then appropriately distributed into 6-well plates. The cells undergoing exponential growth were subjected to a 24-hour treatment with PP extract. The following formulas were used to determine the cells' post-treatment Plating Efficiency (PE) and Survivability Fraction (SF):

$$PE = \text{no. of colonies formed} / \text{no. of cells seeded} \times 100\%$$

$$SF = \text{no. of colonies formed after treatment} / \text{no. of cells seeded} \times PE$$

## 3. Results

MTT assay: The results of the MTT experiment demonstrated a reduction in cell viability dependent on the dosage in all prostate cancer cell lines when treated with extracts from pomegranate juice and peel. Table 1 displays the IC50 values and level of selectivity that correspond to each other. The results of the MTT experiment indicated that ALVA-41 cells responded to the extract in a manner that was similar to that experienced by typical fibroblasts, as shown by the IC50 values rising from around 106 µg/mL after 24 hours to roughly 163 µg/mL after 48 hours. Several other cancer cell lines showed increased sensitivity to the PP extract. The IC50 value for ARCaP cells, as determined at the 24-hour time, was around 65 µg/mL. However, at the 48-hour time, IC50 values were nearly twice as high, with a value of approximately 140 µg/mL, perhaps indicating a decrease in the extract's potency. An escalation in extract toxicity was found in LNCaP and NCI-H660 cells when subjected to extended incubation periods. In the case of LNCaP cells, there was a decrease in the IC50 value from approximately 70 µg/mL at 24 hours to about 39 µg/mL at 48 hours. However, for NCI-H660 cells, a less pronounced decrease in the IC50 value was seen, with values of approximately 43 µg/mL and 36 µg/mL at 24 and 48 hours, respectively [Figure 1].

The SI was ascertained by contrasting how cancer and normal cells responded to the peel of the pomegranate extract. [Table 1] displays the comparison's outcomes. All cell lines had SI values over two at the 24-hour point, indicating that the extract had a significant selectivity towards cancer cells. After 48 hours, the proliferation index remained elevated for NCI-H660 and LNCaP cells, with values of around 6 and 5, respectively [Figure 2a] and [Figure 2b]. This finding suggests that the extracts employed in the study had inhibitory properties against cell growth.

Table 1. Pomegranate peel extract's cytotoxic effects on cancer cell lines

| Cell line | IC50 ± SEM (24 hrs) | IC50 ± SEM (48 hrs) | SI 24hrs | SI 48hrs |
|---|---|---|---|---|
| ALVA-41 | 106.57 ± 1.72 | 163.57 ± 2.24 | 1.32 ± 0.02 | 1.01 ± 0.01 |
| ARCaP | 65.15 ± 1.09 | 139.75 ± 0.64 | 2.98 ± 0.06 | 1.46 ± 0.01 |
| NCI-H660 | 42.92 ± 2.16 | 36.29 ± 1.23 | 3.98 ± 0.26 | 6.05 ± 0.12 |
| LNCaP | 69.85 ± 2.99 | 39.15 ± 1.25 | 2.34 ± 0.09 | 5.02 ± 0.15 |

SEM- Standards Error of Mean, SI-Selectivity Index

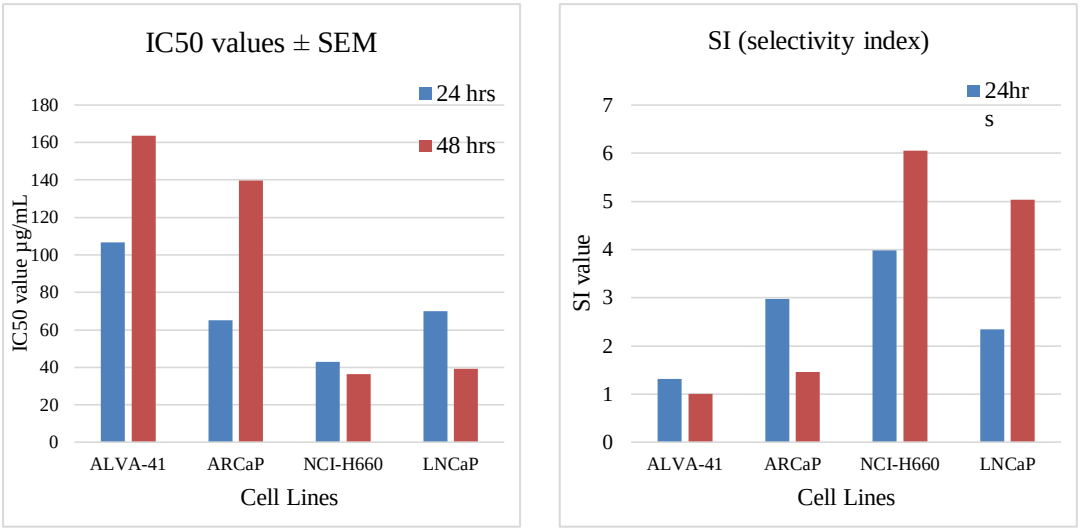


Figure 1. Pomegranate peel extract's cytotoxic effects on cancer cell lines

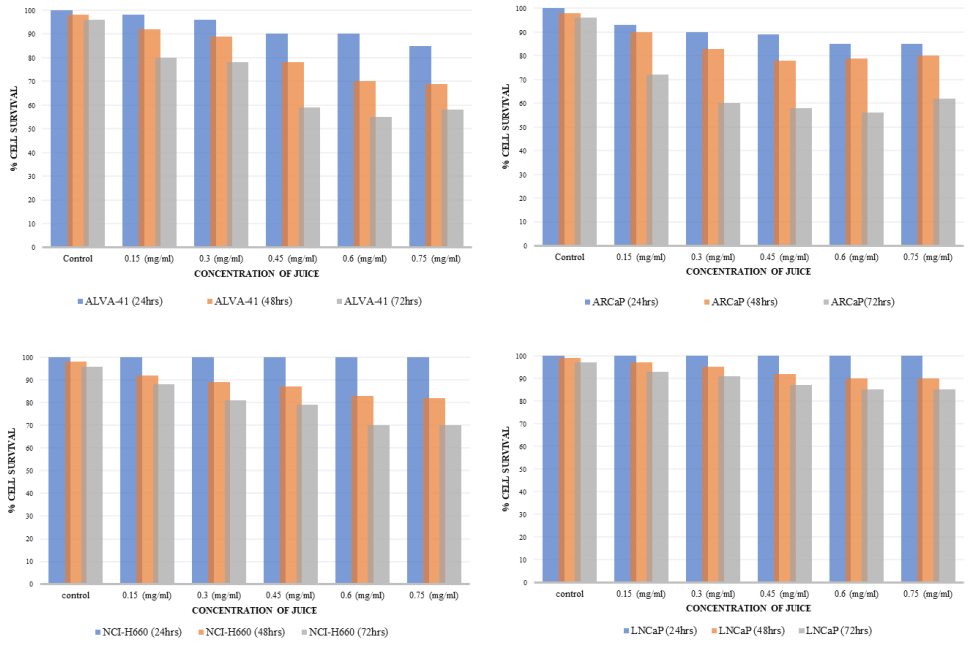


Figure 2a. Pomegranate juice's antiproliferative properties on every cell line

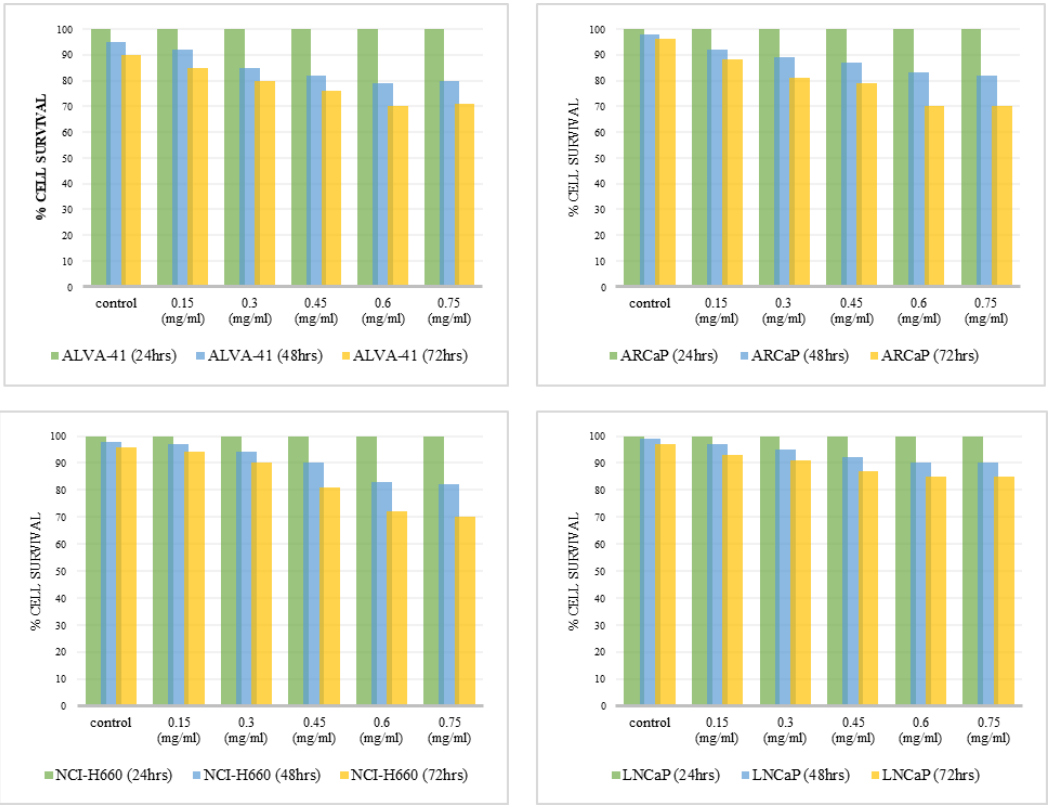


Figure 2b. Antiproliferative effects on cells of a) Pomegranate juice and b) Peel extract

Wound healing experiment: The wound healing test experiment provided evidence that applying pomegranate extracts resulted in a notable inhibition of the migratory capabilities of prostate cancer cells. The experimental group, consisting of treated cells, exhibited a decreased rate of wound closure in comparison to the control group. This observation implies that applying pomegranate extracts may hinder the migration of cancer cells, a critical process in metastasis development.

Clonogenic assay: In the colony formation experiment, it was shown that applying pomegranate extracts resulted in a significant decrease in the capacity of prostate cancer cells to form colonies [Figure 3] and [Figure 4]. The cells that underwent treatment had a reduced number and smaller size of colonies in comparison to the control group. This study's findings suggest that using pomegranate extracts may inhibit the clonogenic capacity of prostate cancer cells.

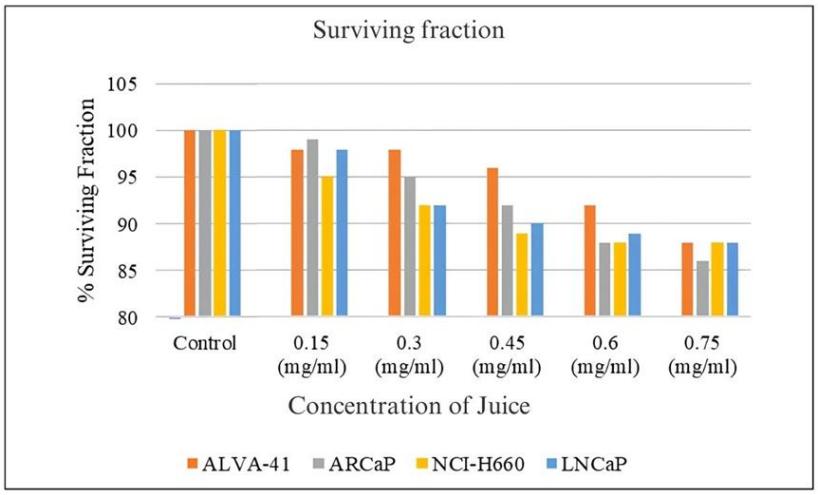


Figure 3. Results of a Clonogenic assay evaluating the survival fraction on

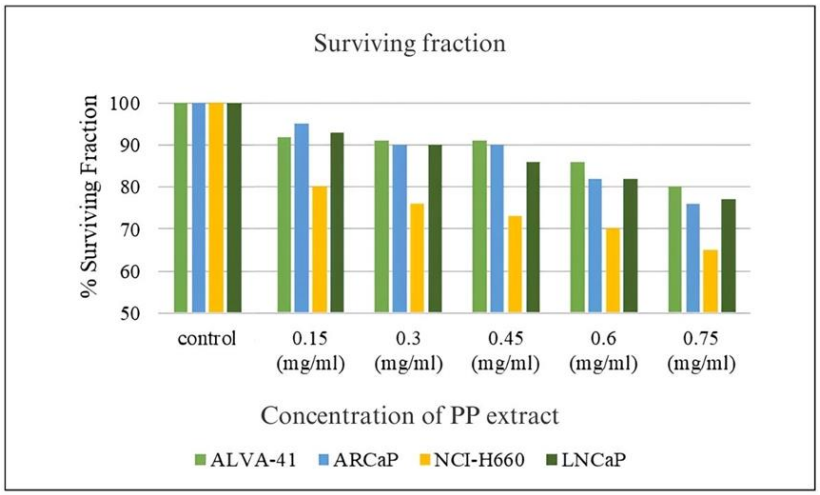


Figure 4. Results of a Clonogenic assay evaluating the survival fraction on a) Pomegranate juice and b) PP extract

## 4. Discussion

The results of this investigation show pomegranate juice's potential and peel extracts as inhibitory agents against prostate cancer progression. Pomegranate extracts significantly affected three critical cancer hallmarks: proliferation, migration, and colony formation. These results align with previous research suggesting the anti-cancer properties of pomegranate-derived compounds, such as punicalagin, ellagic acid, and anthocyanins [18][19][20][21][22].

The inhibition of proliferation observed in this study is particularly promising, as uncontrolled cell growth is a hallmark of cancer. The reduction in cell viability after 72 hours of treatment suggests that pomegranate extracts may interfere with the cell cycle, induce apoptosis, or inhibit growth signaling pathways in prostate cancer cells. Furthermore, a study conducted by Seidi et al. [23], it was revealed that the viability of PC-3 cells decreased by 81.4% when exposed to pomegranate seed extracts at concentrations of up to 5 μg/ml. Chaves et al. [24] also demonstrated that the scratch area in DU-145 and PC-3 cells exhibited sustained preservation when treated with extracts derived from pomegranate juice and peel. The suppression of migration is essential in the context of cancer metastasis, as the ability of cancer cells to migrate and invade surrounding tissues is a significant determinant of cancer progression and patient prognosis. Pomegranate extracts' ability to slow the migration of prostate cancer cells indicates their potential to limit the spread of the disease [25][26].

Colony formation inhibition is another critical aspect of cancer progression. Cancer cells with clonogenic potential can give rise to new tumors, leading to disease recurrence and worsening prognosis. By impeding colony formation, pomegranate extracts may lessen the chance of cancer developing and returning [24][25][27].

The potential anti-cancer effects of pomegranate extracts are diverse and may encompass the regulation of many molecular pathways. Possible processes include control over the cell cycle's evolution, apoptosis activation, and disruption of signaling pathways linked to proliferation and migration. Furthermore, it is worth noting that antioxidants in pomegranate components may play a role in their ability to combat cancer by reducing the harmful effects of oxidative stress, a process intricately connected to the start and development of cancer.

## 5. Conclusion

The inhibitory effects of pomegranate juice and peel extracts on colony formation, migration, and proliferation in lines of prostate cancer demonstrate the direct influence of these extracts on the advancement of prostate cancer. These findings imply that pomegranate extracts may be helpful as supplemental prostate cancer treatment agents. Because pomegranate compounds can target several aspects of cancer growth, they are attractive candidates for further investigation and potential therapeutic usage.

It's important to acknowledge the study's limitations despite the positive results. The in vitro nature of the studies offers an early insight into how pomegranate extracts affect prostate cancer cells. More in vivo studies and clinical trials are needed to verify the favorable effects of pomegranate extract in the treatment of prostate cancer. Furthermore, it is imperative to identify and conduct a more thorough investigation of the particular bioactive chemicals accountable for the reported outcomes. It is also essential to investigate the possibility of drug interactions and the ideal doses for therapeutic use.

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