RESEARCH PAPER

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

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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 a range of health ailments, including cancer. The current study set out to look at the direct effects of the juice of pomegranates and peel extract on the advancement of prostate cancer. We evaluated their capacity to impede prostate cancer cell lines' colony formation, motility, and proliferation as cancer cells. To achieve this, the MTT test was used to measure the drop in the survival of cells that followed a PP extract injection. Prostate cancer cells' ability to form colonies and their ability to be inhibited from migrating were 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, Anticancer, Antioxidants, Pomegranate, Cytotoxicity
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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 variations in social, environmental, and genetic variables. The prevalence of prostate cancer, along with the slow progression of several tumours and the possibility of negative 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 the survival rates, these approaches often come with various side effects and limited efficacy during a later stage of the illness[5]. Consequently, interest in is rising for exploring alternative and adjunctive therapies, especially those that harness the potential of natural compounds[6-8]. The field of ethnopharmacology has facilitated the exploration of traditional medical practices, leading to the identification of several therapeutic compounds. For instance, digitoxin, a cardiac glycoside that has been extracted from *Digitalis purpurea* (foxglove). This substance has demonstrated potential in the management of cardiovascular disorders and has also been comprised into 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] . To reduce patient suffering and cut expenses associated with today's costly therapies, it is crucial to identify innovative ways for cancer treatments. Toxicology to the body's healthy cells is one of the main drawbacks of chemotherapy, resulting in significant side effects like fatigue, alopecia, anaemia, and increased susceptibility to bruising and bleeding, among other adverse consequences[8]. The pharmacological properties and broad structural diversity of plant-based substances and their byproducts have demonstrated a lot of potential for the development of chemotherapy drugs[9]. FDA is the United States Food and Drug Administration authorized the semi-synthetic derivative with a natural taxoid, called cabacitaxel (Jevtana®), 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 are being sold as dietary supplements a lot lately due to their possible health advantages. For instance, through upregulating the expression for CYP7B1, the gene that encodes for the enzyme known as oxysterol 7 alpha-hydrolase, it has been discovered that ingestion of

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pomegranate extract lowers levels of liver and blood cholesterol. The documented cholesterol-lowering benefits of pomegranate extract are further enhanced by its downregulation of SREBP1, a gene that is 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[10].

Pomegranate is known for its unique combination of bioactive compounds, including polyphenols, flavonoids, and anthocyanins, primarily found in both the juice and peel[11-12] . These substances have demonstrated to possess antioxidant, anticancer and anti-inflammatory properties, making them an attractive candidate for cancer therapy and prevention[13-14].Our goal in this research was to look at the direct impact of pomegranate juice and peel extracts on prostate cancer progression, particularly

Materials and Methods

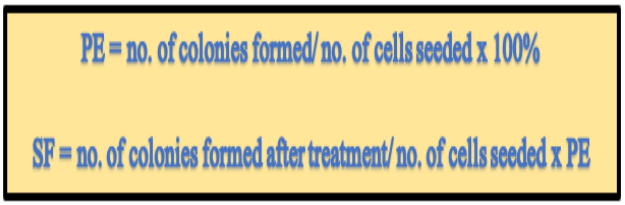
Pomegranate Juice and Peel Extraction: The ripe fruits of native pomegranate plants were harvested from the rural region in order to get unaltered, naturally occurring fruit. The peel was manually separated and subsequently air-dried for a period of 4 to 6 days at room temperature. Prior to extraction, the dried peel was crushed using a laboratory mill. Subsequently, a powdered form of pomegranate peel weighing 50 g was subjected to extraction using a 50% ethanol solution. The extraction process was carried out in an ultrasonic bath for a duration of 40 minutes, maintaining a temperature of 60ºC[15]. 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 standardised procedures. In summary, the fruits underwent a cleaning process, followed by the extraction of juice by pressing and centrifugation techniques.

Cell Culture: ALVA-41, NCI-H660, ARCaP (also known as MDA PCa 1), and LNCaP are human cancer cell lines that were 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) we 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 subsequent to the injection of PP extract. After being exposed to varying concentrations of extracts, the cells were incubated for 24, 48, and 72 hours. A microplate reader was used to measure the absorbance at 570 nm in wavelength. According to Lopez-Lázaro (2015), the degree of selectivity, sometimes referred to as the selectivity index (SI), is the ratio of a sample's hazardous concentration to its effective bioactive concentration[16]. Divided by the IC50 values obtained from the malignant cell line (A549) for every experiment, the mean of the IC50 readings for the normal 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 time 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 process of trypsinization 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. Using the following formulae, the plating efficiency (PE) as well as survivability fraction (SF) of the cells after treatment were calculated:



Results

MTT Assay: The findings of the MTT experiment demonstrated a reduction in cell viability that was dependent on the dosage in all prostate cancer cell lines when treated with extracts from both pomegranate juice and peel. Table 1 displays the IC_{50} 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 with 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. A number of other cancer cell lines showed increased sensitivity to the PP extract. The IC_{50} value for ARCaP cells, as determined at the 24-hour time point, was around 65 µg/mL. However, at the 48-hour time point, IC_{50} values were nearly twice as high with a value of approximately 140 µg/mL, perhaps indicating a decrease in the potency of the extract. 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 IC_{50} 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 IC_{50} value was seen, with values of approximately 43

µg/mL and 36 µg/mL at 24 and 48 hours, respectively (Figure 1).

By contrasting how cancer and normal cells responded to the peel of the pomegranate extract, the SI was ascertained. Table 1 displays the comparison's outcomes. All cell lines had SI values more than 2 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 2). 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***

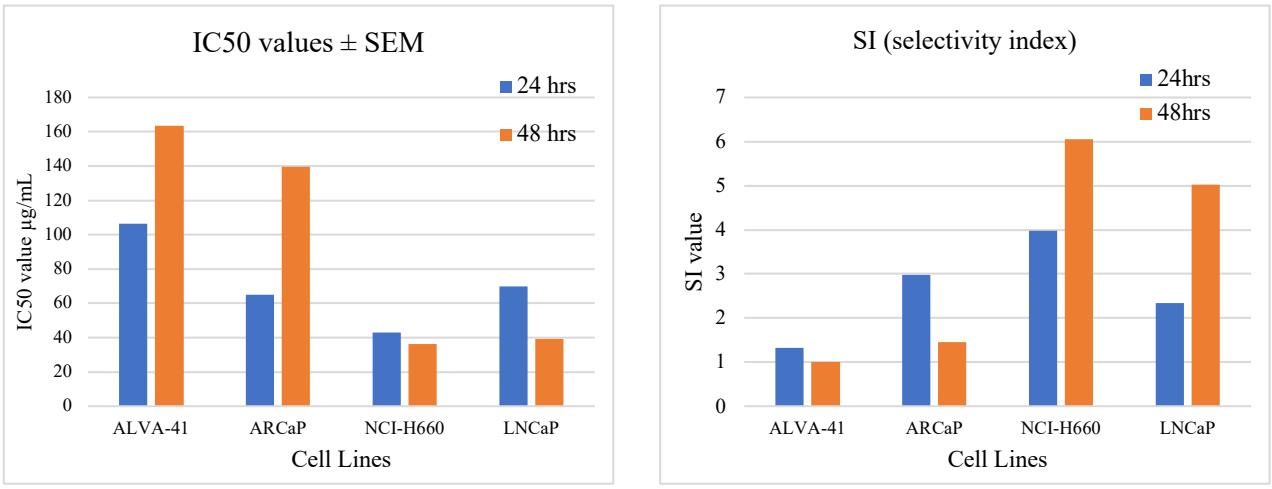


Figure 1. Pomegranate peel extract's cytotoxic effects on cancer cell lines

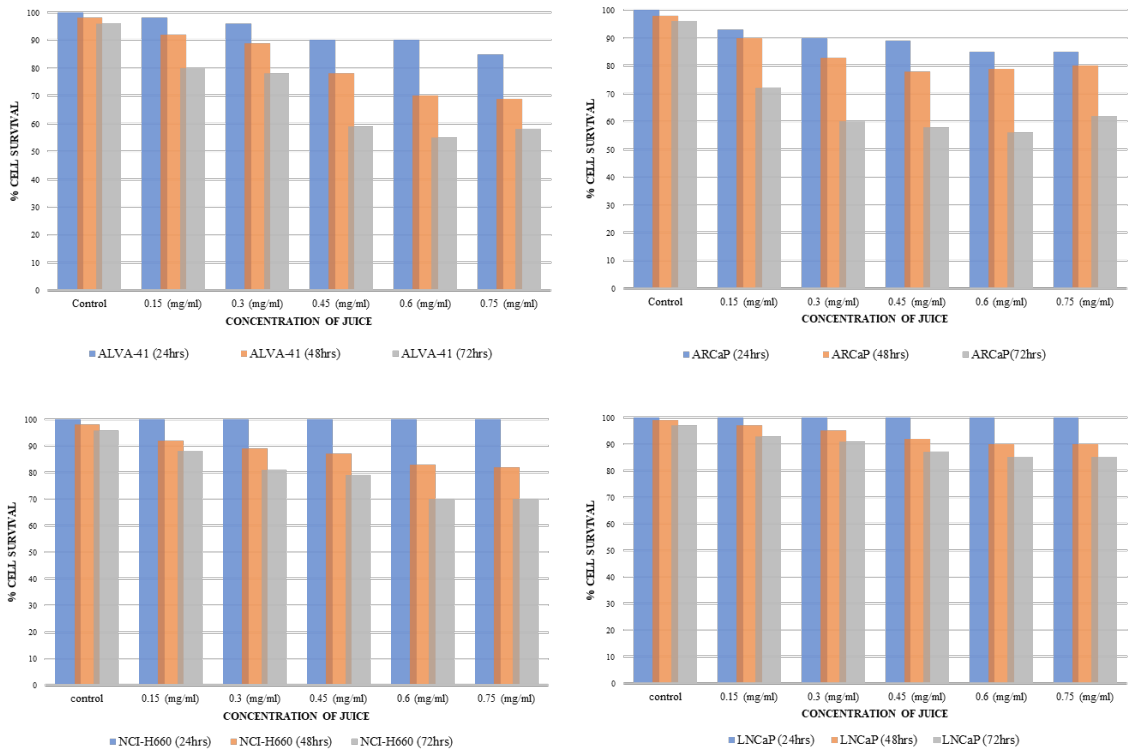
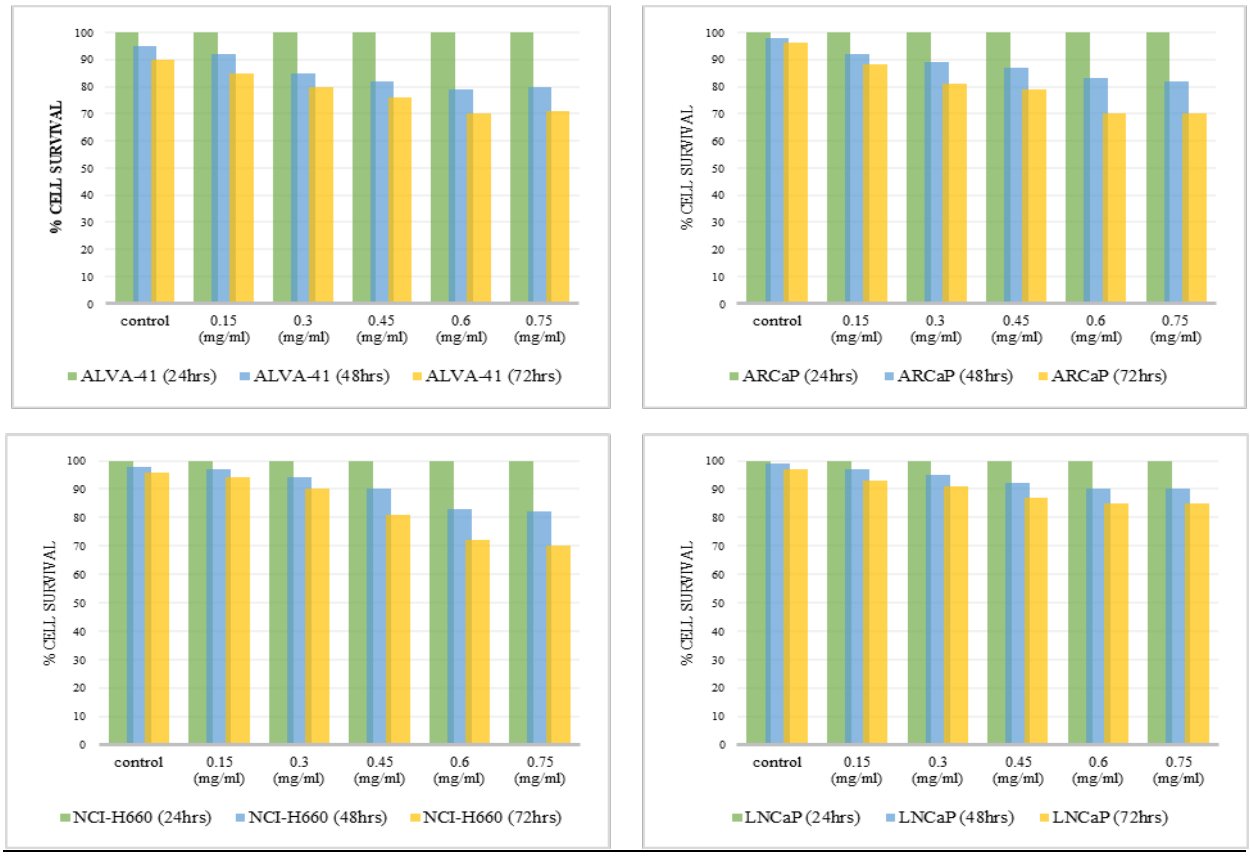


Figure 2. a. Pomegranate juice's antiproliferative properties on every cell line



b)**Figure 2. b). Antiproliferative effects on cell of a) Pomegranate juice b) Peel extract**

Wound healing experiment: The experiment utilising the wound healing test provided evidence that the application of 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 the application of pomegranate extracts may hinder the migration of cancer cells, a critical process in the development of metastasis.

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Clonogenic Assay: In the colony formation experiment, it was shown that the application of pomegranate extracts resulted in a significant decrease in the capacity of prostate cancer cells to form colonies (Figure 3). The cells that underwent treatment had a reduced number and smaller size of colonies in comparison to the control group. The findings of this study suggest that the use of pomegranate extracts may inhibit the clonogenic capacity of prostate cancer cells

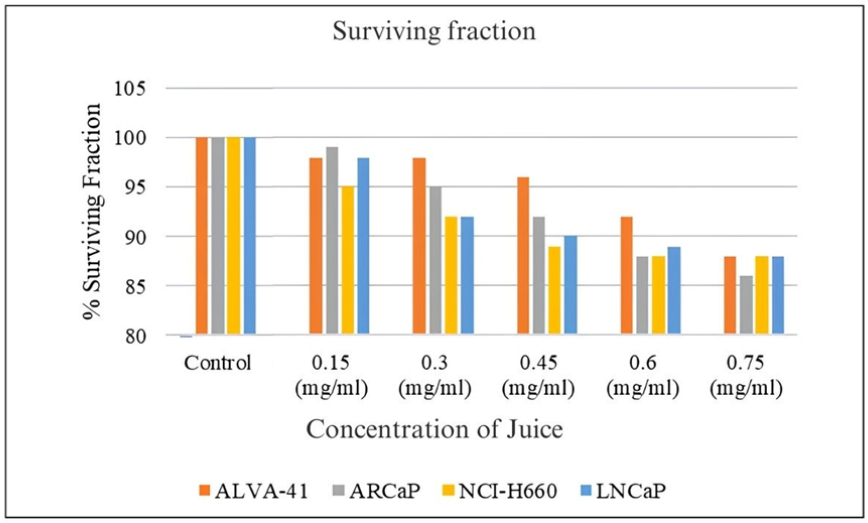

Figure3: Results of a Clonogenic assay evaluating the survival fraction on

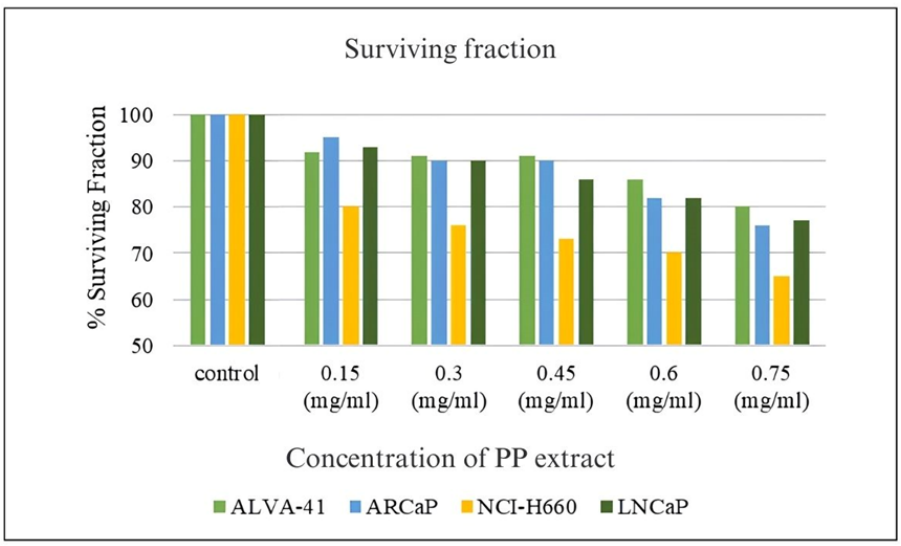

Figure 3: Results of a Clonogenic assay evaluating the survival fraction on a) Pomegranate juice b) PP extract

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Discussion

The results of this investigation show pomegranate juice's potential and peel extracts as inhibitory agents against prostate cancer progression. Pomegranate extracts exhibited significant effects on three critical cancer hallmarks: proliferation, migration, and colony formation.

These results align with previous research suggesting the anticancer properties of pomegranate-derived compounds, such as punicalagin, ellagic acid, and anthocyanins[17-21].

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 signalling pathways in prostate cancer cells. Furthermore, a study conducted by Seidi et al. (2016), 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[22,23]. Additionally, the study by Chaves et al. (2020) demonstrated that the scratch area in DU-145 and PC-3 cells exhibited sustained preservation when subjected to treatment with extracts derived from pomegranate juice and peel[24]. 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 down the migration of prostate cancer cells indicates their potential to limit the spread of the disease[25-26].

The 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 anticancer effects of pomegranate extracts are believed to be diverse and may encompass the regulation of many molecular pathways. Potential processes may encompass, control over the evolution of the cell cycle activation of apoptosis, and disruption of signalling pathways linked to proliferation and migration. Furthermore, it is worth noting that the presence of antioxidants in pomegranate components may potentially 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.

Conclusion

The inhibitory effects of pomegranate juice as well as 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 useful 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.

Limitations

It's important to acknowledge the study's limitations even though the results are positive. The in vitro nature of the studies offers an early insight of how pomegranate extracts affect prostate cancer cells. To confirm the beneficial effects of pomegranate extract in the treatment of prostate cancer, further research is required, including in vivo investigations and clinical trials. 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 important to investigate the possibility of drug interactions and the ideal doses for therapeutic use.

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Authors' contributions

Author contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

Declaration of Conflicts of Interests

Author declares that they have no conflict of interest.

Ethics approval and consent to participate

Not applicable as no experiment was conducted on any animal or human.

Availability of data and materials

Not Applicable

Use of Artificial Intelligence

Not applicable

Declarations

Authors declare that all works are original and this manuscript has not been published in any other journal..

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REFERENCE

1. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, et al. Global Cancer Observatory: Cancer Today. Lyon (FR): International Agency for Research on Cancer; 2023. Available from: https://gco.iarc.fr/today. Accessed 13 Oct 2023.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424.
3. Hayes JH, Barry MJ. Screening for prostate cancer with the prostate-specific antigen test: a review of current evidence. JAMA. 2014;311(11):1143–1149.
4. Fridlender M, Kapulnik Y, Koltai H. Plant-derived substances with anti-cancer activity: from folklore to practice. Front Plant Sci. 2015;6:799.
5. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin. 2017;67(1):7–30.
6. Nobili S, Lippi D, Witort E, Donnini M, Bausi L, Mini E, et al. Natural compounds for cancer treatment and prevention. Pharmacol Res. 2009;59(6):365–378.
7. Boik J. Natural compounds in cancer therapy. Princeton (MN): Oregon Medical Press; 2001.
8. Majolo F, Delwing LKOB, Marmitt DJ, Bustamante-Filho IC, Goettert MI. Medicinal plants and bioactive natural compounds for cancer treatment: important advances for drug discovery. Phytochem Lett. 2019;31:196–207.
9. Pan L, Chai HB, Kinghorn AD. Discovery of new anticancer agents from higher plants. Front Biosci

(Schol Ed). 2012;4:142–156.
10. Koltai T, Reshkin SJ, Baltazar F, Fliegel L. Prostate cancer metabolism: from biochemistry to therapeutics. London: Academic Press; 2021.
11. Viladomiu M, Hontecillas R, Lu P, Bassaganya-Riera J. Preventive and prophylactic mechanisms of action of pomegranate bioactive constituents. Evid Based Complement Alternat Med. 2013;2013:789764.
12. Ranjha MMAN, Shafique B, Wang L, Irfan S, Safdar MN, Murtaza MA, et al. A comprehensive review on phytochemistry, bioactivity and medicinal value of bioactive compounds of pomegranate (Punica granatum). Adv Tradit Med. 2021;21:1–21.
13. Prakash CVS, Prakash I. Bioactive chemical constituents from pomegranate (Punica granatum) juice, seed and peel: a review. Int J Res Chem Environ. 2011;1(1):1–18.
14. Karasawa MMG, Mohan C. Fruits as prospective reserves of bioactive compounds: a review. Nat Prod Bioprospect. 2018;8:335–346.
15. Akhtar S, Ismail T, Fraternale D, Sestili P. Pomegranate peel and peel extracts: chemistry and food features. Food Chem. 2015; 174:417–425.
16. López-Lázaro M. Two preclinical tests to evaluate anticancer activity and to help validate drug candidates for clinical trials. Oncoscience. 2015;2(2):91–98.
17. Adhami VM, Khan N, Mukhtar H. Prevention of cancer with pomegranate and pomegranate anthocyanins. In: Seeram NP, Heber D, editors. Berries and cancer prevention. New York: Springer; 2011. p. 209–226.
18. Bishayee A, Darvesh AS. Pomegranate-derived constituents as inducers of cell death: implications in cancer prevention and therapy. In: Garg AK, Aggarwal BB, editors. Natural compounds as inducers of cell death. Vol 1. Dordrecht: Springer; 2012. p. 33–47.
19. Syed DN, Afaq F, Mukhtar H. Pomegranate-derived products for cancer chemoprevention. Semin Cancer Biol. 2007;17(5):377–385.
20. Miguel MG, Neves MA, Antunes MD. Pomegranate (Punica granatum L.): a medicinal plant with myriad biological properties. J Med Plants Res. 2010;4(25):2836–2847.
21. Panth N, Manandhar B, Paudel KR. Anticancer activity of Punica granatum (pomegranate): a review. Phytother Res. 2017;31(4):568–578.
22. Seidi K, Jahanban-Esfahlan R, Abasi M, Abbasi MM. Antitumoral properties of Punica granatum seed extract in different human cancer cells. Asian Pac J Cancer Prev. 2016;17(3):1119–1122.
23. Lugito NPH, Djuwita R, Adisasmita A, Simadibrata M. Blood pressure lowering effect of Lactobacillus-containing probiotic. Int J Probiotics Prebiotics. 2022;17(1):1–13. Available from: https://www.nchpjournals.com/admin/uploads/ijpp2641-7197v17n1-1-13.pdf
24. Chaves FM, Pavan ICB, da Silva LGS, de Freitas LB, Rostagno MA, Antunes AEC, et al. Pomegranate juice and peel extracts inhibit proliferation, migration and colony formation of prostate cancer cell lines and modulate Akt/mTOR/S6K signaling. Plant Foods Hum Nutr. 2020;75:54–62.
25. Deng Y, Li Y, Yang F, Zeng A, Yang S, Luo Y, et al. Pomegranate peel extract induces apoptosis and impairs metastasis in prostate cancer cells. Biomed Pharmacother. 2017;93:976–984.
26. Wang L, Alcon A, Yuan H, Ho J, Li QJ, Martins-Green M. Cellular and molecular mechanisms of pomegranate juice-induced anti-metastatic effects on prostate cancer cells. Integr Biol (Camb). 2011;3(7):742–754.
27. Keta OD, Deljanin M, Petković V, Zdunić G, Janković T, Živković J, et al. Pomegranate (Punica granatum L.) peel extract: potential cytotoxic agent against different cancer cell lines. Rec Nat Prod. 2020;14(5):326–339
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