American Journal of Biochemistry and Biotechnology

Original Research Paper

Antiproliferative Potential of Pomegranate Peel Extract in MDA-MB Breast Cancer Cell Lines

[1]Manal Asif Hamdulay, [1]Fatema Yusuf Singaporewala, [2]Grisilda Vidya Bernhardt, [3]Pooja Shivappa, [1]Norhan Elsayed Mohammed and [1]Fariha Yasmin

[1]Department of Basic Sciences, RAK College of Medical Sciences, Ras Al Khaimah Medical and Health Sciences University, RAK, United Arab Emirates
[2]Department of Biochemistry, RAK College of Medical Sciences, Ras Al Khaimah Medical and Health Sciences University, RAK, United Arab Emirates
[3]Department of Biophysics, Manipal School of Life Sciences, Manipal Academy of Higher Education, Manipal, India

Article history
Received: 19-11-2024
Revised: 29-01-2025
Accepted: 04-02-2025

Corresponding Author:
Grisilda Vidya Bernhardt
Department of Biochemistry, RAK College of Medical Sciences, Ras Al Khaimah Medical and Health Sciences University, RAK, United Arab Emirates
Email: grisilda@rakmhsu.ac.ae

And

Pooja Shivappa
Department of Biophysics, Manipal School of life sciences, Manipal Academy of Higher Education, Manipal, India poojacs77@gmail.com

Abstract: Nutraceuticals have gained recognition as adjuvant therapies in cancer treatment due to their non-toxic and protective properties. Among various natural products, pomegranate peel with high antioxidant capacity and rich polyphenol content suggest potential anticancer properties. Previous studies have shown promising anticancer effects of other parts of the pomegranate. The purpose of this research is to compare the effectiveness of Pomegranate Peel Extract (PPE) on triple-negative breast cancer with that of oestrogen receptor-positive breast cancer. We assessed PPE's anticancer potential using MTT, Superoxide Dismutase (SOD) activity and Glutathione Peroxidase (GPX) on the MDA-MB-231 and MCF-7 cell lines for breast cancer. Initial cytotoxicity assays demonstrated favourable results in both cell lines, prompting further evaluation of antioxidant activity, particularly in MDA-MB-231. The cytotoxicity tests showed positive results following PPE treatment in the MDA-MB-231 and MCF-7 breast cancer cell lines. Following these initial results, the SOD and GPX assays revealed a significant antioxidant activity in the MDA-MB-231 cell line, indicating PPE's potential role in reducing oxidative stress in these cells. These results suggest that PPE exhibits anticancer activity and antioxidant properties that may be beneficial in targeting difficult-to-treat breast cancers, particularly MDA-MB-231. According to the study's findings, PPE shows promise as an antioxidant and anticancer agent, particularly when used to treat triple-negative breast cancer. The cytotoxicity, SOD activity and GPX assay results show promise for PPE as a preventive or adjuvant treatment for breast cancer. To completely comprehend its mechanics and therapeutic effects in clinical settings, further investigation is necessary.

Keywords: Breast Cancer, Pomegranate, Cytotoxicity, Sod Activity, GPX Activity, Triple Negative Breast Cancer

Introduction

Cancer continues to rank among the world's leading causes of mortality, making it a severe issue. One out of four people is in danger, and its prevalence has sharply grown (Roy and Saikia, 2016). This rise is accelerated by factors like diseases, biological predispositions and living environments (Emmons and Park, 2000). As the most frequent cancer detected in 2020, breast cancer was linked to 685,000 deaths and 2.3 million new cases; by 2040, those numbers are predicted to skyrocket to over 3 million cases and 1 million fatalities (Arnold *et al*., 2022). There is no denying that breast cancer is one of the leading causes of death and incidence worldwide, and there is currently no effective treatment for it.

Primarily affecting women over 50, its prevalence highlights the significant global health concern. Genetic mutations, especially in BRCA1 and BRCA2, contribute to 5-10% of cases, making prevention crucial (Bennett *et al*., 1999). Breast cancer is classified into four molecular subtypes, each influencing prognosis and treatment. The prognosis and treatment are influenced by the distinct features of each subtype. The 5-year survival rate for metastatic Triple-Negative Breast Cancer (TNBC) is

12%, while the 5-year survival rate for Metastatic Breast Cancer (MBC) is just 29%. These low survival rates emphasize how urgently alternative treatment options for metastatic breast cancer, with a focus on TNBC, need to be investigated (Burguin *et al*., 2021).

Breast cancer treatment faces significant challenges, including therapy resistance and recurrence, with around 30% of early-stage cases returning and often leading to metastasis (Pan *et al*., 2017). Current treatments have limitations due to their toxic profiles, making long-term administration problematic. Radiation therapy, while common, can cause cardiotoxicity and shows resistance in advanced cases. Chemotherapy also has serious side effects, such as cardiomyopathy, secondary cancers, premature menopause, infertility and psychological impacts. These issues highlight the urgent need for alternative treatment strategies that improve efficacy and reduce adverse effects (Burguin *et al*., 2021).

In recent years, researchers have increasingly prioritized disease prevention as a key management strategy. It is currently believed that making dietary changes could prevent up to two-thirds of deaths caused by cancer (Moga *et al*., 2021). Considering the substantial side effects of current treatments, exploring complementary therapies is highly beneficial (Greenlee *et al*., 2017). The goal is to incorporate non-toxic alternatives alongside conventional methods rather than replace them, and nutraceuticals are well-suited for this purpose (Ko *et al*., 2021).

Pomegranate peels, which have potential anticancer effects, are one of the valuable resources that are frequently thrown away as garbage in the United States. Pomegranate Peel Extract (PPE) has the potential to be used as a natural cancer treatment in addition to addressing the environmental effects of food waste (Hajimahmoodi *et al*., 2013; Ferrante *et al*., 2023). Numerous recent studies have emphasized the nutritional advantages of the pomegranate's seeds, arils and peel (Mokbela and Mokbela, 2019). Polyphenols such as anthocyanins, tannins, flavonoids, phenolic acids and lignans are abundant in the peel, which makes up almost half of the fruit's weight, in comparison to other sections (Banerjee *et al*., 2011). Since the peel contains more ellagitannins, proanthocyanidins, polysaccharides and flavonoids than the pulp, it has a higher antioxidant capacity (Sreeja *et al*., 2012).

Pomegranate Peel Extract (PPE) contains delphinidin and quercetin, which have been seen to suppress breast cancer cell growth and boost the efficacy of current treatment options. Many studies have indicated that high flavonoid intake, including quercetin, is associated with a reduced breast cancer risk (Bagheri *et al*., 2014). PPE's phytochemicals have increased potential for developing into breast cancer treatments and can further enhance the efficacy of chemopreventive drugs such as tamoxifen (Ozbay and Nahta, 2011). Additionally, PPE exhibits properties similar to SERMs on breast cancer cell lines and shows multiple beneficial effects, including inhibiting aromatase, reducing inflammation and regulating key cancer pathways (Kim *et al*., 2022). Given these advantages, pomegranate peel is a highly promising candidate for overcoming current breast cancer treatment challenges.

Disturbance in metabolic signalling pathways, oxidative stress, mineral and vitamin metabolism, calcium signalling and oxidative stress are associated with breast cancer (Forterre *et al*., 2020; Bhat *et al*., 2012). Therefore, in cancer treatment, the dual nature of antioxidants, both as protective agents against oxidative stress and as enhancers of therapeutic efficacy, is becoming increasingly significant. Higher metabolic rates in cancer cells result in increased oxidative stress, and the generation of an excess of ROS can cause mutations and encourage the growth of tumours (Gorrini *et al*., 2019; Bernhardt *et al*., 2021). Severe inflammation marked by elevated CRP and other inflammatory markers along with oxidative stress is exposure to toxic substances associated with cancers, including breast cancers (Pierce *et al*., 2009; Pinto *et al*., 2022; Bernhardt *et al*., 2009). Antioxidants are essential for scavenging reactive oxygen species, which are known to cause oxidative stress and DNA damage in cells, hence contributing to the advancement of cancer and resistance to treatment (Reuter *et al*., 2010; Bernhardt *et al*., 2012). Antioxidants play a key role in cancer prevention and treatment by reducing ROS, which can lessen cellular damage in both cancer and healthy cells (Trachootham *et al*., 2009; Luis *et al*., 2019).

Our study explores the anticancer potential of Pomegranate Peel Extract (PPE) by analyzing its cytotoxic and antiproliferative effects on two cell lines of breast cancer: MDA-MB-231, which represents estrogen-independent Triple-Negative Breast Cancer (TNBC) and MCF-7, which represents estrogen-dependent breast cancer. This dual approach aims to explore PPE’s efficacy across a spectrum of breast cancer subtypes, from hormonally driven to the most aggressive forms. We will employ cell viability and cytotoxicity assays, specifically the MTT assay, to evaluate PPE’s impact on cancer cell growth and sensitivity at varying concentrations. Additionally, antioxidant properties will be assessed using Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPX) assays, providing insights into PPE’s potential to mitigate oxidative stress and its therapeutic value.

By addressing critical questions about whether PPE exhibits anticancer properties, possesses a robust antioxidant and ant proliferative profile and demonstrates dose-dependent effects, this study aims to determine its viability as a preventive, complementary, or adjuvant therapy PPE's importance in developing breast cancer treatment options is highlighted by its capacity to lessen the cytotoxic side effects of traditional treatments and to act as an affordable, sustainable alternative for managing breast cancer recurrence.

Materials and Methods

The PPE extract used for the study was purchased from WonderLand Herbs, originating from Bellingham, Washington, USA. And contained 60% polyphenols. This extract is GMP-certified and manufactured under strict quality control measures to ensure purity and efficacy.

Cell Lines

MCF-7 and MDA-MB-231 human breast cancer cells were cultured in Dulbecco's Modified Eagle Medium with 3.7 g/L sodium bicarbonate, 10% complete media and 1% penicillin/streptomycin added.

The cell cultures were maintained in a humidified atmosphere with 5% CO_2 at 37°C. The assays were conducted three times, with each experiment containing three replicates (Sreeja *et al*., 2012).

Viability Assay (MTT)

The proliferation of the cells was assessed using the Methyl Thiazol Tetrazolium (MTT) assay. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) is enzymatically reduced to MTT-formazan, which is an indicator of cell viability (Luis *et al*., 2019).

Based on initial standardization, cells were planted in 96-well plates at a density of 1×10^5 cells per well. They were then incubated for 24 h at 37°C with 5% CO_2. Pomegranate peel extract was prepared in DMSO to create a stock solution at 10 mg/mL, and serial dilutions were made in complete media, with DMSO added to facilitate dye solubilization. The concentration ranges for MDA-MB-231 cells were 30.7-92.25 mg/L, and for MCF-7 cells, 100-3.12 mg/L. The concentration range of PPE (100-3.12 mg/L) was selected based on preliminary optimization experiments and existing literature. Initial cytotoxicity screening identified this range as suitable for observing both cytotoxic and sub-cytotoxic effects in MDA-MB breast cancer cell lines, allowing for a clear dose-response relationship. Previous studies have reported similar ranges for polyphenolic extracts, including punicalagin and ellagic acid. Concentrations >100 mg/L were excluded due to nonspecific toxicity, while those <3.12 mg/L showed negligible effects, ensuring physiological relevance and experimental feasibility. Treatments were applied for 24 h. The assay included a vehicle control, a negative control and Doxorubicin as a positive control. The formation of blue formazan was measured by absorbance at 600 nm. Results were averaged and normalized to the control. The following formula was used to calculate the cytotoxicity percentage (Moradi-Gharibvand *et al*., 2022):

$$\%\ Cytotoxicity = (100 \times (Control - sample)/Control$$

Superoxide Dismutase (SOD) Assay

SOD activity is defined as the enzyme level at which the inhibition ratio of SOD in the reaction system reaches 50%, corresponding to one unit of SOD activity. SOD assay was performed to assess antioxidant capacity and oxidative stress.

SOD activity was measured using an ELISA approach with the Elabscience kit. This method involves a competitive ELISA, where plates are pre-coated with SOD. SOD from the cell lysate competes with the fixed SOD on the plates for binding sites on biotinylated detection antibodies specific for SOD. The optical density of the resulting reaction was measured with an ELISA plate reader.

Following a 24-hour exposure to PPE at IC50 concentrations, the cells were lysed using a lysis buffer. The results were expressed as a percentage of SOD activity inhibition after the supernatant was extracted for the SOD assay in accordance with the kit's instructions. Cells were trypsinized, lysed and washed with PBS after treatment to provide cell lysates that could be used to measure SOD activity (Moradi-Gharibvand *et al*., 2022).

Glutathione Peroxidase Assay

The amount of GPx in 1 mg of protein is the enzyme activity that catalyzes the consumption of 1 micromolar per litre (μM/L) of GPx substrate.

GPx was performed using an ELISA reader, and Elabscience Glutathione Peroxidase (GSH-Px) Activity Assay Kit was used for this assay. After being collected, the cells were lysed in a cold lysis buffer and rinsed with cold PBS. To get rid of debris, the lysates were centrifuged at 10,000 g for 15 min at 4°C. For analysis, the supernatant was gathered. Cell lysates were combined with reaction buffer, GSH and NADPH in accordance with the manufacturer's instructions for the test. Hydrogen peroxide was added to start the reaction, and GPx activity was evaluated by measuring the drop in absorbance at 340 nm. Protein extraction/estimation for x, expressed in milligrams of protein (Moradi-Gharibvand *et al*., 2022).

Statistical Analysis

The results of all experiments are expressed as a percentage of control ± Standard Error (S.E.) of triplicate determinations. Data analysis was carried out using GraphPad Prism V8.1. The Shapiro-Wilk test confirmed data normality, and an unpaired t-test was applied to compare various GAE concentrations with the control and Doxorubicin within the same cell line. Statistical significance was set at $p<0.05$.

Results

An MTT assay was conducted to assess the impact of PPE on the proliferation of MDA-MB-231 and MCF-7 breast cancer cell lines.

The results showed induced cytotoxicity upon treatment with PPE. MDA-MB showed highest cytotoxicity of 52.73% at a concentration of 79.95 mg/L of PPE. MCF-7 showed more promising results of 81.92% cytotoxicity at a concentration of 25 mg/L mg of PPE. These findings suggest a dose-dependent antiproliferative effect of PPE on both the cell lines; it also indicated that PPE has a time and concentration-dependent cytotoxic impact on the cancer cell. A detailed explanation is shown in Tables (1-2), Figs. (1-2).

MCF-7 cells were treated with concentrations ranging from 3.125-100 mg/L. Highest toxicity on cells was seen at 25 mg/L. MDA -MB cells were treated with concentrations ranging from 30.7-92.25 mg/L.

Impact of Pomegranate Peel Extract on the Antioxidant Enzymes Activity of MCF-7 and MDA-MB-231 Human Breast Cancer Cells

SOD Activity

SOD assay was conducted on DMSO, PPE extract and Doxorubicin and compared with the control. The highest increase in SOD activity was seen by Doxorubicin, followed by PPE. A 50% statistically significant increase was noted in SOD activity in the PPE-treated group compared to the control. Cells that were treated with PPE showed an average SOD activity of 1.93 units, and Doxorubicin demonstrated a comparable increase in SOD activity (Table 3 and Fig.).

The unpaired t-test revealed that there was no significant difference in SOD activity between PPE and Doxorubicin ($p = 0.3$), suggesting similar effects of both treatments. However, a significant difference was observed between PPE and the control group ($p = 0.0437$), indicating that PPE significantly increased SOD activity compared to the untreated cells. This suggests that PPE enhances antioxidant defence mechanisms, while Doxorubicin and PPE show comparable effects in this specific context.

Table 1: Impact of pomegranate peel extract on cytotoxicity in MCF-7 cell lines

Concentrations (mg/L)	Inhibition #((± SD)
3.12	49.019±3.54
6.25	66.91±1.64
12.5	75.848±0.44
25	81.59±0.40
50	69.86±0.22
100	72.77±2.8

All values are expressed as Mean ± Standard Deviation

Table 2: Impact of pomegranate peel extract on cytotoxicity in MDAMB-231 cell lines

Concentrations (mg/L)	Inhibition #((± SD)
30.7	-2.603±3.5
36.9	24.4116±3.75
43	7.2789±4.21
49.2	13.4672±1.98
55.3	17.2412±1.5
61.5	34.87669±2.45
67.65	15.44368±2.82
73.8	37.179±2.99
79.95	52.734±1.96
86.1	44.6791±2.9
92.25	34.7547±4.3

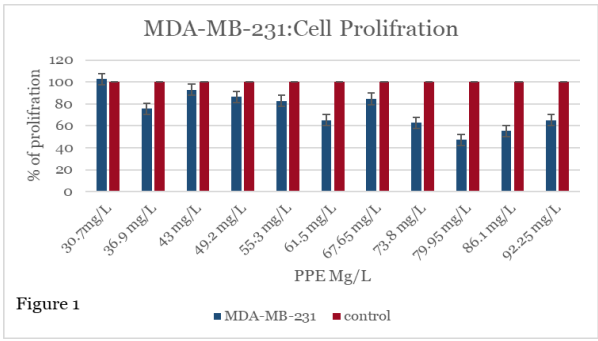

Fig. 1: Effect of Pomegranate Peel Extract on Cell Proliferation in MDA-MB cell lines: Proliferation was assessed using the MTT assay

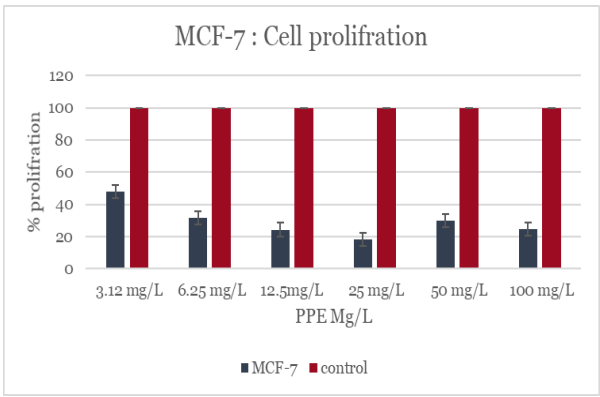

Fig. 2: Effect of Pomegranate Peel Extract on Cell Proliferation in MCF-7 cell lines: Proliferation was assessed using the MTT assay

GPx Activity

GPx assay was conducted on DMSO, PPE extract and Doxorubicin and compared with the control. TheThe group that showed the most significant reduction in GPx activity was PPE, followed by Doxorubicin. Cells treated with PPE showed GPx activity averaging approximately 347 units per milligram protein.

The unpaired t-test revealed a significant difference in GPx activity between PPE and Doxorubicin (p = 0.0169), indicating that the treatments affected GPx activity differently. Additionally, a highly significant difference was observed between PPE and the control group (p<0.001), with PPE substantially increasing GPx activity compared to the control (Table 3 and Fig. 3). This suggests that PPE has a pronounced effect on enhancing antioxidant enzyme activity, and its impact differs significantly from that of Doxorubicin.

Table 3: Effects of pomegranate peel extract and Doxorubicin on SOD and GPx activity in MDA-MB-231 breast cancer cell lines

	Control	DMSO	PPE-Extract	Doxo
SOD activity %	0.964±0.098	1.33±0.22	1.91±0.32*	2.233±0.26
GPx activity Units per milligram per protein	914±9.93	835±10.80	347.66±17.32#	412.3±10.20

All values are expressed as Mean ± Standard Deviation; statistical analysis was performed using an unpaired t-test, comparing each treatment condition with the control. Statistical significance was defined as p≤0.05. * significant difference in values when compared to control, #statistical significance was seen between PPE and control & PPE (pomegranate) peel extract and Doxo (Doxorubicin)

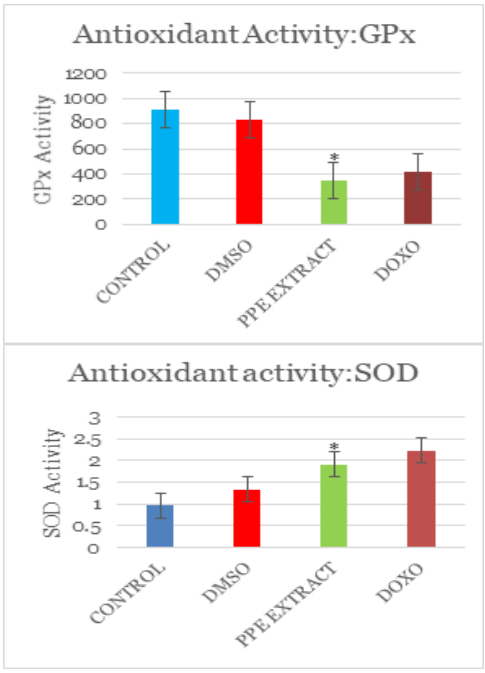

Fig. 3: Antioxidant capacity of pomegranate peel extract assessed via SOD activity and GPx assays in MDA-MB cell lines, with statistical comparisons to the PPE conducted using an unpaired T-test. Significance was set up at ≤0.05; in SOD activity, statistical significance (* p = 0.0437) was seen between PPE and control. For GPx, statistical significant (**p<0.001; *p = 0.0169) was seen between PPE and control & PPE (pomegranate) peel extract and Doxo (Doxorubicin)

Discussion

Breast cancer remains a significant global health challenge, with Triple-Negative Breast Cancer (TNBC) representing one of the most aggressive subtypes due to its limited treatment options and poor prognosis. In this context, the exploration of natural compounds with anticancer properties offers a promising avenue for developing safer and more effective therapies. This study explores the ant proliferative potential of PPE in MDA-MB-231 breast cancer cell lines, a widely used model for TNBC research.

Our findings revealed a time-dependent cytotoxic effect of PPE, with the highest cytotoxicity observed at concentrations of 79.97 mg/L for MDA-MB-231 cells and 25 mg/L for MCF-7 cells after 24 h. This correlates with the extract's phenolic content, which is known for its antioxidant properties.

The differing results of PPE on the cytotoxicity on these cell lines might be because estrogen-dependent MCF-7 cells involve a unique response to PPE sensitivity through estrogen signalling pathway, whereas estrogen-independent MDAMB-231 cells require higher PPE concentration due to distinct cellular mechanisms or resistance pathways. Variations in metabolism, PPE uptake, and genetic and molecular characteristics of these cells can also explain the observed differences. The decreased proliferation observed in MDAMB-231 cell lines significantly highlights the potential of PPE in targeting TNBC. To further understand PPE's action on the general resistance of MDA-MB-231 cells, we focused exclusively on these estrogen-independent cells for their applicability in treating more aggressive breast cancer types. An article demonstrated increased SOD activity by PPE in MCF-7 cell lines, supporting our decision to investigate MDA-MB-231 cells further (Luís *et al*., 2023).

Our study also included additional analyses of PPE's antioxidant enzymatic activity, particularly focusing on SOD activity and glutathione systems. The observed increase in SOD activity in cells treated with PPE suggests that the enzyme's upregulation may be a defensive response. PPE-induced cytotoxicity is due to the induction of oxidative stress via releasing oxidants such as reactive oxygen species. The increase in ROS subsequently upregulated SOD activity in the treated cells representing an adaptive response to counteract these potentially damaging species. The enhancement of SOD activity can be interpreted as a protective cellular mechanism activated by the oxidative environment triggered by PPE. This mechanism might be crucial for minimizing damage while maximizing the antiproliferative effects of the treatment (Vidya Bernhardt *et al*., 2024; Sarmiento-Salinas *et al*., 2019). In our study, the effect of PPE caused a decrease in GPx activity, thereby decreasing the oxidative stress, which correlated with another study, thereby highlighting the chemotherapeutic properties of

PPE. Luís *et al*., 2023; Bagheri *et al*., 2018), the decrease in GPx activity due to PPE treatment induces oxidative stress in breast cancer cells, thereby contributing to the anticancer effect. The reduction in GPx activity reflects the cells' diminished ability to neutralize oxidative damage, which might enhance the cytotoxic effects of the treatment on the cancer cells (Moga *et al*., 2021; Luís *et al*., 2023; Shivappa and Bernhardt, 2022; Ahmed *et al*., 2022).

The balance between cytotoxicity and cellular defence highlights ROS's dual role in promoting and inhibiting cancer progression, suggesting that careful alteration of this balance could enhance the therapeutic efficacy of PPE (Sarmiento-Salinas *et al*., 2019). Our study emphasizes PPE's role in modulating cell viability, which is key to developing less toxic cancer therapeutic alternatives. One of the common side effects of conventional chemotherapy is oxidative stress. The observed increase in SOD activity in cells treated with PPE suggests that its application could mitigate oxidative stress, potentially improving the therapeutic index of anticancer treatments (Moga *et al*., 2021). The cost-effective potential of natural sources as a therapeutic option is not highlighted much due to insufficient scientific evidence. A diet rich in fruits and vegetables is emerging as a compelling strategy to significantly reduce cancer risk. Thus, the integration of antioxidants such as those from pomegranate peel extract into cancer treatment strategies, along with considerations of other factors like single nucleotide polymorphism that affect individual responses to oxidative stress, offers a promising avenue for both enhancing cancer therapy and reducing its toxic effects (Moga *et al*., 2021; Bernhardt *et al*., 2021; Sarmiento-Salinas *et al*., 2019). This comprehensive approach could potentially lead to improved therapeutic outcomes in breast cancer management, either as a preventive measure or as a complementary treatment to conventional therapies.

Pomegranate Peel Extract (PPE) exhibits its anticancer effects on breast cancer, particularly TNBC, through multiple mechanisms. It upregulates ICAM-1, enhancing cell adhesion and reducing metastatic potential while decreasing the expression of MMP9, an enzyme critical for ECM degradation and tumour invasion. PPE also downregulates fibronectin, which is associated with cancer progression and metastasis and suppresses VEGF, a key driver of angiogenesis required for tumour growth and metastasis. These effects collectively inhibit cancer cell migration, invasion and metastasis. PPE's modulation of these pathways highlights its potential as an effective antimetastatic agent in breast cancer therapy (Bagheri *et al*., 2018).

One of the challenges in targeting MDA-MB-231 cells lies in their molecular profile, particularly the frequent presence of p53 mutations. These mutations are associated with resistance to apoptosis, a characteristic that complicates treatment strategies. Despite this, our study demonstrates PPE's ability to induce oxidative stress and cytotoxicity in these cells, providing valuable insights into its potential as a therapeutic agent for TNBC.

Overall, this study emphasizes the significance of PPE as a natural, cost-effective therapeutic option with the potential to enhance breast cancer treatment. By modulating key antioxidant systems and inducing oxidative stress, PPE represents a promising candidate for further research, either as a standalone agent or in combination with conventional therapies, to improve therapeutic outcomes in TNBC management.

Future research should focus on validating the findings in vivo models to assess the systemic effects and bioavailability of PPE, along with its safety profile. Detailed exploration of molecular pathways, such as NF-κB, STAT3 and p53-independent apoptosis mechanisms, can provide deeper insights into its anticancer effects. Investigating the potential of PPE in combination with conventional therapies could enhance efficacy and reduce toxicity. Additionally, standardized methods for PPE preparation and nanoparticle-based delivery systems could improve specificity and therapeutic outcomes.

Conclusion

In conclusion, this study highlights the significant therapeutic potential of PPE in combating breast cancer. Our research demonstrated that PPE exhibits potent antiproliferative effects on both estrogen-dependent MCF-7 and estrogen-independent MDA-MB-231 breast cancer cell lines. The results from MTT assays reveal that PPE's cytotoxic effects are concentration- and time-dependent, with notable reductions in cell viability and proliferation, particularly at higher doses.

The observed increase in SOD activity and decrease in GPx activity suggest that PPE enhances cellular antioxidant defences, which may contribute to its antiproliferative effects. This dual mechanism combining direct cytotoxicity with oxidative stress modulation underscores PPE's potential as a powerful agent in breast cancer therapy.

Our findings align with and extend previously done studies validating the efficacy of PPE and supporting its role in targeting aggressive breast cancer forms (Bernhardt *et al*., 2021; Nair *et al*., 2011; Seidi *et al*., 2016). The observed differences in PPE's effects on MCF-7 versus MDA-MB-231 cells highlight the complexity of its action and the need for further research to fully elucidate its mechanisms and optimize therapeutic strategies.

Given the positive results observed, PPE demonstrates significant potential as an adjunct therapy in breast cancer treatment, offering benefits for both estrogen-dependent and estrogen-independent subtypes. The absence of adverse side effects further underscores its promise as a safe and effective addition to existing cancer treatment regimens. Continued research will be essential to fully

understand its therapeutic potential and maximize its use in combating diverse breast cancer subtypes.

This study adds to the growing research on natural compounds in cancer therapy. Further investigations are needed to figure out the molecular mechanisms and confirm the therapeutic potential of pomegranate peel extracts in cancer treatment.

Acknowledgement

I acknowledge my professors for their guidance, my college for their support, and my university for providing the resources and environment necessary for this research.

Funding Information

This study received no external funding.

Author's Contributions

All authors equally contributed in this work.

Ethics

We received ethical approval from RAKMHSU and RAK-REC.

Competing Interests

All the authors declare that there are no conflicts of interest.

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