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ANTI-PROLIFERATIVE POTENTIALS OF PLANT EXTRACTS AND AMYGDALIN AGAINST THE MCF-7 BREAST CANCER CELL LINE

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Abstract

Plant-derived chemicals are being investigated as possible anticancer medicines, since breast cancer is still a major worldwide health concern. This study investigated the anti-proliferative effects of amygdalin and a few plant extracts (green tea, pomegranate peel, ginger, garlic, and turmeric) on MCF-7 breast cancer cells. MCF-7 and MCF-10A as normal cells were treated, with extracts produced through a standardized drying process at a concentration of 50 µg/mL, while amygdalin was measured at both 50 µg/mL and 0.5 mg/mL. Through lowering cell viability, inducing apoptosis (caspase-3/7 up to 264.55%), increasing reactive oxygen species (ROS) (up to 148.63%), lowering Adenosine Triphosphate (ATP) levels (as low as 50.12%), and elevating Lactate dehydrogenase (LDH) release, the tested plant extracts, particularly garlic (*Allium sativum* L.) (40.63 %), turmeric (*Curcuna longa* L.) (47.58%), and ginger (*Zingiber officinale* Roscoe) (44.85%) showed significant anti-proliferative effects on MCF-7 breast cancer cells. High-dose amygdalin (0.5 mg/mL) exhibited the strongest responses across all parameters (viability 31.11%, caspase 315.45%, ROS 220.15%, ATP 40.07%). Green tea (*Camellia sinensis*) and pomegranate (*Punica granatum* L.) extracts showed moderate effects, reducing nitric oxide (NO) generation (3.68 µM, 2.54 µM); slightly changing ROS (80.05%, 96.54%) and total protein levels (68.45 µg/mg, 94.85 µg/mg) while retaining lower cytotoxicity to MCF-10A cells, respectively. Overall, the findings highlighted the potential of the selected plant extracts, as natural anti-cancer medicines by demonstrating that, they exhibit selective cytotoxicity and apoptotic potential. Those results were on equal with or better than the commercial amygdalin, particularly accurate for garlic and turmeric. More research is needed to fully examine the mechanisms and therapeutic efficacy of natural bioactive components in the treatment of cancer and to confirm their potential as safe and efficient anti-cancer medications.

Key words: Breast cancer; MCF-7; Anticancer; Medicinal plants; Cytotoxicity; Reactive oxygen species

Introduction

Cancer is a serious problem, as it is still one of the major causes of death worldwide. Diseases, biological predispositions, and living conditions are some of the reasons that exacerbate the increase in incidence (Marta *et al.,* 2025). There is no effective treatment for breast cancer, which is undeniably one of the principal causes of death and the high incidence of the disease. It primarily affects women over 50 years of age, and thus has drawn attention to the serious worldwide health issue. Since 5-10% of cases are caused by genetic abnormalities, particularly in breast cancer genes 1 and 2 (BRCA1 and BRCA2). The main symptoms of breast cancer as a genetic syndrome are, unchecked cell growth and inhibited cell death (Liu *et al.,* 2022). About 30% of early-stage cases recur and frequently result in metastases. Treatment for breast cancer is loaded with difficulties, including therapy resistance and recurrence. Due to their hazardous characteristics, current therapies have limitations that make long-term

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administration challenging. Despite being widely used, radiation therapy can be cardiotoxic, and, in more severe cases, it can exhibit resistance. Serious adverse effects of chemotherapy include infertility, cardiomyopathy, secondary cancers, early menopause, and psychological effects. These problems show how urgently innovative therapeutic approaches that increase effectiveness and reduce side effects are needed (Hamdulay *et al.,* 2025). One of the most common and dangerous cancers in the world is still breast cancer. Drug resistance, toxicity, and non-selective harm to healthy cells frequently restrict the clinical efficacy of conventional treatments including chemotherapy, radiation, and hormonal therapy, despite the fact that they have improved patient outcomes. As a result, there is increasing interest in investigating safer and more potent alternative treatment agents, especially bioactive chemicals produced from plants that may have anticancer properties. Nowadays expensive and highly side-effect-prone treatment options, including hormone therapy, radiotherapy, chemotherapy, and surgery, frequently lower quality of life. Plant-based medications could provide a viable therapy and preventative approach (Rezaeidian *et al.,* 2024). As a crucial management approach, disease prevention has gained more attention from researchers in recent years. Up to two-thirds of cancer-related deaths are thought to be avoidable with dietary modifications (Moga *et al.,* 2021). Investigating complementary therapy is very advantageous given the significant adverse effects of existing medications. The purpose is to incorporate non-toxic substitutes and nutraceuticals rather than replace conventional methods.

The active yellow pigment in turmeric is (*Curcumin*), a hydrophobic polyphenol that is extracted from the *Curcuma longa* L. plant rhizome, and has been shown to have potential anti-cancer effects. In a number of cancer types, such as stomach, breast, and colon cancer, curcumin has shown anti-tumor properties (Bahadar *et al.,* 2024). Studies have examined ginger (*Zingiber officinale* Roscoe) for its anti-inflammatory properties, while shogoal and zingerone, vanilloids such as 6-gingerol and 6-paradol, have been linked to the anticancer properties (Afshin *et al.,* 2022). Through downregulating MMP-2 and MMP-9 metalloproteinases, which are recognized to have a role in inducing cancer cell invasion, gingerol treatment of breast cancer cells inhibited cell proliferation via the presence of 10-gingerol, which inhibited mitogen-induced Aka Protein Kinase B (AKT) activation, and Epidermal Growth Factor Receptor (EGFR) expression (Zraikat *et al.,* 2024). Garlic (*Allium sativum L.*) has been demonstrated to have therapeutic benefits and is widely used as an immunomodulator and natural treatment for a variety of illnesses, including cancer. Numerous bioactive substances, including diallyldisulfide, diallyltrisulfide, ajoene, allicin, and alliin, have been identified in garlic. Through the caspase-dependent/-independent pathway, allicin demonstrated a chemopreventive impact on gastric cancer by reducing the development of cancer cells at the G2/M phase (Mandal *et al.,* 2019). Human leukemic cells undergo apoptosis due to ajoene, a secondary metabolite of garlic, which stimulates peroxide production and activates caspase-3 and caspase-8. One of the valuable resources that is routinely discarded as waste in manufacturing is pomegranate peels, which may have anticancer properties. Delphinidin and quercetin, which are found in pomegranate peel extract (PPE), have been shown to inhibit the proliferation of breast cancer cells and increase the effectiveness of existing treatment strategies. The phytochemicals found in PPE have a greater chance of becoming therapies for breast cancer and can improve the effectiveness of chemopreventive medications as tamoxifen (Ozbay & Nahta, 2011). Furthermore, PPE has several positive effects, such as lowering inflammation, blocking aromatase, and controlling important cancer pathways, and it displays characteristics similar to those of Selective Estrogen Receptor Modulators (SERMs) on breast cancer cell lines of garlic, which stimulates peroxide production and activates caspase-3 and caspase-8 (Kim *et al.,* 2002). Green tea (*Camellia sinensis*) is one of the world healthiest drinks. The most prevalent catechin in green tea is epigallocatechin-3-gallate (EGCG), which may have anti-tumorigenic properties by modifying interleukin in gastric cancer cells. It can inhibit the NFκB signaling pathway, prevent metastasis triggering apoptosis, and several signaling pathways, including p38, Extracellular signal-Regulated Kinases (ERK1/2), and AKT (Roy *et al.,* 2020). Due to the capacity to alter important cellular processes implicated in the development of cancer, chemicals produced from plants have garnered growing interest as possible anticancer medicines (Ozbay & Nahta, 2011). Through mechanisms as oxidative stress modulation, mitochondrial malfunction, and activation of caspase signaling pathways, bioactive phytochemicals have been shown in numerous studies to suppress tumor cell proliferation by causing apoptosis (Kim *et al.,* 2002). Based on the abundance in phenolics, flavonoids, and sulfur-containing chemicals with established antioxidant and pro-apoptotic properties, the chosen plant extracts were examined in this context (Roy *et al.,* 2020).

The kernels of several fruits, such as peaches, apricots, apples, and plums, as well as the cores of pears and the pits of cherries, naturally contain amygdalin (D-mandelonitrile-β-Gentiobioside), a cyanogenic diglucoside. It can be well known as B-17 with a commercial name as Laetril. When mixed with other substances such as benzaldehyde and hydrocyanic acid, amygdalin has a synergistic impact that causes breast cancer cells to die. An enzyme called β-glucosidase can break down amygdalin, releasing glucose, benzaldehyde, and hydrogen cyanide. Hydrogen cyanide has the ability to cause cyanide toxicity, which kills cancer cells. Amygdalin has been shown to have cytotoxic and apoptosis-inducing properties in a variety of cancer cell types, it was included in this investigation as a pertinent comparator for assessing the anticancer potential of the examined plant extracts. However, another enzyme called rhodanese, which is found in healthy tissues only and not in cancerous ones, appears to be able to detoxify cyanide and shield healthy tissues in the process (Christodoulou *et al.,* 2022). Figure 1 presents the 3D molecular structure of amygdalin.

The anticancer efficacy of some selected plant extracts such as turmeric, ginger, garlic, pomegranate peel, green tea, and the commercial supplement amygdalin against MCF-7 breast cancer cells was investigated. Lactate dehydrogenase (LDH) assays and MTT measurements of cell viability were used to quantify cytotoxicity. Trypan blue exclusion distinguished between living and deceased cells, confirming cell viability. Apoptosis was evaluated by caspase-3/7 activity, whereas oxidative stress was indicated by nitric oxide levels as determined by the Griess technique, besides evaluating their impact on MCF-7 breast cancer cells' levels of Adenosine Triphosphate (ATP), total protein, and intracellular reactive oxygen species (ROS) production.

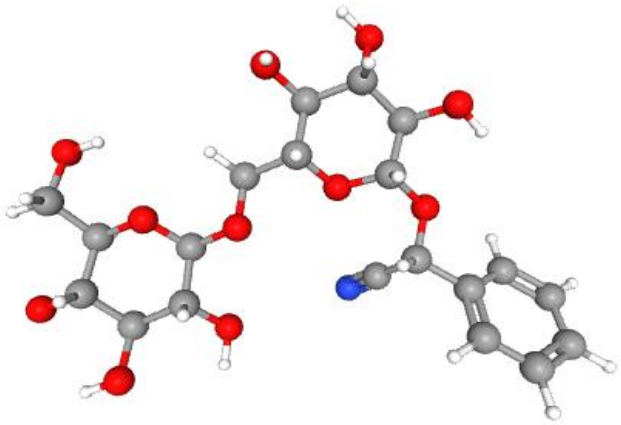

Fig. 1. 3D molecular structure of amygdalin. Reference: (PubChem database (CID: 656516).

Material and Methods

Sample preparation: The dried green tea (*Camellia sinensis*) leaves from (Baja, Panda Hyper 20010, Taif) and fresh samples of pomegranate (Punica granatum L.) peel, ginger (Zingiber officinale Roscoe), garlic (*Allium sativum* L.), and turmeric rhizomes (*Curcuna longa* L.) were purchased from a local market in Taif, Saudi Arabia. Amygdalin was purchased from Sigma-Aldrich (A6005, St. Louis, Missouri, USA). The plant materials were prepared for laboratory analysis, according to standard drying and other processing procedures. After cleaning each sample with distilled water, to get rid of any surface contaminants and further adhering dirt, the samples were allowed to air dry on lint-free tissue paper to get rid of any remaining moisture. For uniform drying operation, the cleaned samples were then uniformly sliced to a thickness of 2-3 mm with a stainless-steel lab knife. Depending on the type of sample, drying was carried out for 48 - 72 hours at 45°C in a hot air oven (Yiheng, Shanghai Ins. Co., Ltd., China) until a consistent weight was reached. The dried samples were ground into a fine powder, using a laboratory grinder (MF-10, Jingxin Co., Ltd., China) and sieved through a 60-mesh stainless steel sieve to achieve uniform particle size. The powdered samples were then stored in labeled, airtight glass containers and kept at room temperature in a dry, dark cabinet until further analysis.

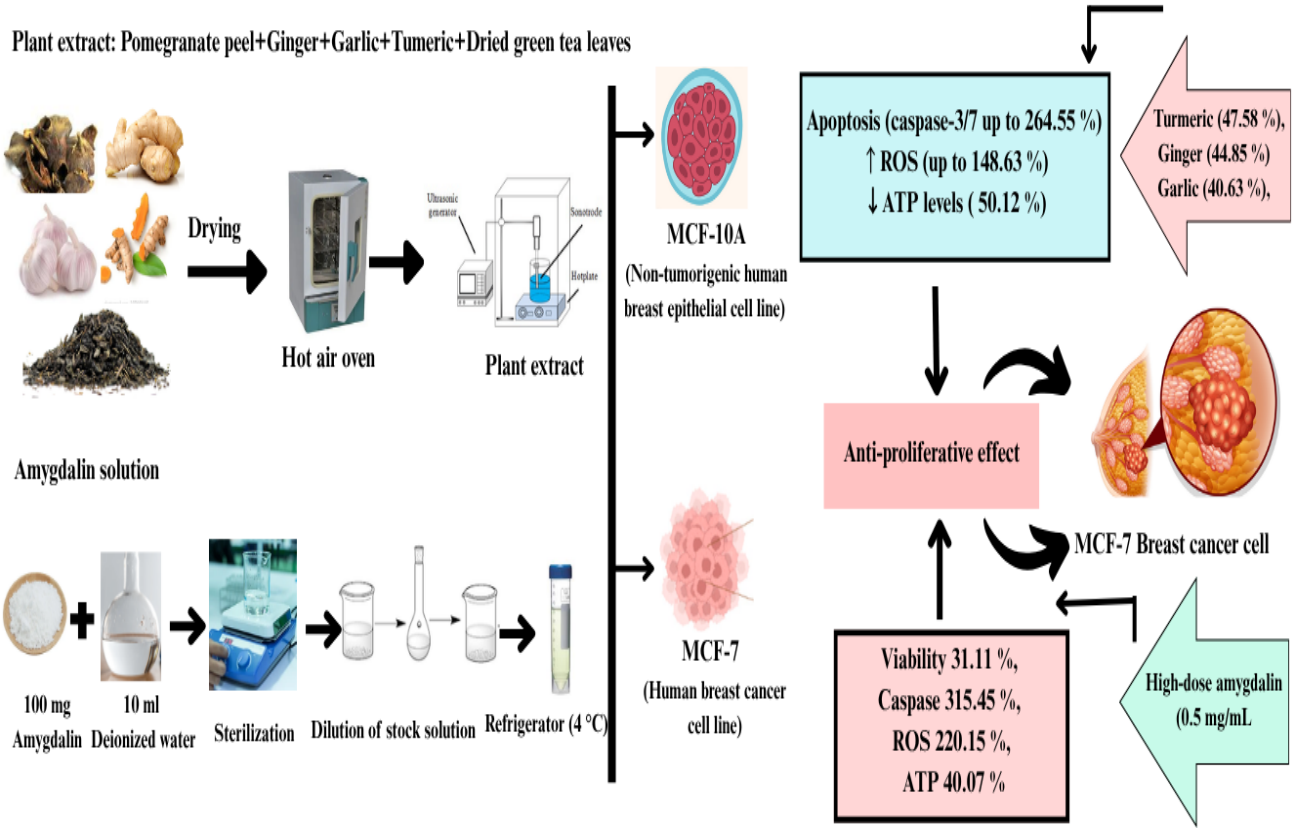

Fig. 2. Summary of the experimental work on of the effect of some selected plant extracts as garlic bulbs, pomegranate peel, green tea leaves, turmeric and ginger rhizomes compared to amygdalin on MCF-10A and MCF-7 human epithelial cell lines.

Sample extraction: The selected samples were carefully weighed (30 g), and the extraction was conducted using the following solvents: distilled water for hydrophilic compounds such as garlic, pomegranate peel, and green tea leaves, and 70% ethanol for lipophilic compounds such as turmeric and ginger. After being exposed to ultrasonic waves (JP 100S Benchtop, Yuhuan, China) for ten minutes, the mixtures were shaken for 24 hours at room temperature (Afshin *et al.,* 2022; Zraikat *et al.,* 2024). In order to improve compound solubility, the plant powder was soaked in the solvents at a 1:10 (w/v) ratio and stirred for 30 minutes at room temperature, then gently heated (50°C) in a water bath. Particulate matter was subsequently removed from the crude extracts by filtering with Whatman No. 1 filter paper, followed by rotary evaporator (YRE 2000E, Gongyi, China) until thick, and kept in amber vials at 4°C. The stock extract was diluted in Dulbecco's Modified Eagle Medium (DMEM) to reach a final concentration of 50 µg/mL using a vortexer (VMX MT, Infitek, China), as established by dry weight yield, in order to prepare the working solution for cell culture treatments. In order to preserve the quality of the bioactive compounds, all extracts were either newly made or used within 24 hours. First, 100 mg of amygdalin was dissolved in 10 mL of sterile deionized water to create a stock solution containing 10 mg/mL. This was followed by the preparation of amygdalin solutions of 50 µg/mL and 0.5 mg/mL. Filter sterilization (0.2 micron) was applied to the stock solution. 50 µL of the stock was diluted with 10 mL of sterile water to obtain 50 µg/mL. 500 µL of stock was diluted to 10 mL for 0.5 mg/mL. The dried extracts were reconstituted in DMEM after solvent evaporation under low pressure. The final treatment media's residual ethanol concentration was kept at 0.1% (v/v), which is often thought to be non-cytotoxic to mammalian cells in culture. The solutions were used immediately and kept at 4°C in light-protected conditions (Christodoulou *et al.,* 2022).

Cell culture: Non-tumorigenic (MCF-10A) and breast cancer (MCF-7) human epithelial cell lines, were purchased from the Shanghai Institute of Biological Sciences, China. These cells were cultured in tissue culture flasks at 37°C with a 5% CO_2 incubator (WJ 3T series, Shanghai, China) under humidified condition (85%) in RPMI-1640 media supplemented with 100 units/mL of penicillin-streptomycin, 0.14% sodium bicarbonate, 0.1 mM sodium pyruvate, and 100 mg/mL streptomycin supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Sigma-Aldrich, Louis, USA) (Kadi *et al.,* 2025). These cells (3×10^4 cells mL^{-1}) were plated, tested, and verified on a regular basis and incubated for 48 hours. Cells at the exponential development phase (70-80% confluency) were used for the experiments. Before their suspension was combined with 15 mL of full medium, they were incubated for 5 minutes. The recovered cells were used for the *In vitro* tests after the cell suspension was centrifuged for 10 minutes at 3,500 rpm. Figure 2 presents the summary of the experimental work on MCF-10A and MCF-7) human epithelial cell lines.

Contamination control: All experimental procedures were carried out aseptically in a laminar flow biosafety cabinet. To guarantee contamination-free cultures, sterile culture media, solvents, and consumables were regularly examined under a microscope. Additionally, every experiment contained suitable solvent controls. Vehicle controls were cells treated with the highest concentration of the solvent used for extract dilution (such as DMSO or distilled water). Solvent concentrations were maintained constant and below cytotoxic levels to guarantee that any effects were exclusively attributable to the tested extracts and not the solvent itself.

Cytotoxicity assay: The MTT (Methyl Thiazol Tetrazolium) assay was used to assess cytotoxicity, which serves as an indicator of cell viability (Qiao *et al.,* 2020). The selected plant extracts (turmeric, ginger, garlic, pomegranate peel, and green tea) were coded as Tur, Gin, Gar, Pom, and G-tea, respectively, at 50 µg/mL in relation to a commercial supplement; also, the amygdalin was tested at both 50 µg/mL and 0.5 mg/mL as positive controls. In order to allow cell adhesion, cells were seeded in 96-well plates with a total of 10^4 cells/well and incubated overnight at 37°C in a humidified environment with 5% CO_2 for 24 hours. Following treatment, each well received 20 µL of MTT reagent, 5 mg/mL of Phosphate-Buffered Saline (PBS), and was incubated for four hours at 37°C. After the medium was carefully removed, 100 µL of DMSO was added to each well to dissolve the formazan crystals that had formed. The cells were kept out of the light and incubated at room temperature for 12 hours. Following the incubation period, the plate was stirred for ten minutes using a horizontal shaker (HY 2B, Jiangsu, China) before being read for the optical density with a microplate reader (RT 2100C, Shenzhen, China) to detect absorbance at 595 nm, using a reference wavelength of 550 to 670 nm. A dose of 0 µg/mL was used as the negative control group. The proportion of cell viability was expressed as a percentage in relation to untreated control cells, and it was determined according to equation 1.

$$\text{Cytotoxicity (\%)} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100 \qquad \text{Eq. 1}$$

Trypan blue exclusion assay: Trypan blue is an azo dye with a negative charge that only interacts with the membranes of cells that were affected (Olofinsan *et al.,* 2025a). Viable cells and non-viable cells in percentage were evaluated using the Trypan Blue exclusion assay. Cell suspensions from the various extracts were first diluted to encourage cell adhesion in order to decrease the cell population. In contrast, cells were seeded in 6-well plates at a density of 1×10^5 cells per well and incubated for 24 hours at 37°C in a humidified incubator with 5% CO_2. Following the treatment, adherent and floating cells were separated by trypsinization, and then centrifuged for five minutes at 1,000 rpm. 20 µL of the cell suspension for each extract was combined with 10 µL of 0.4% Trypan Blue dye and placed on a Countess slide to each side after the cell pellet had been reconstituted in 1 mL of PBS with a pipette in a 500 µL Eppendorf tube. Before

being loaded into a hemocytometer (BK-CC10, Shandong, China), the mixture was allowed to sit at room temperature for two minutes. A cell counter (C100-SE, Shenzhen, China) was used to automatically estimate the number of live and dead cells. Blue-stained cells were regarded as non-viable, whereas cells that did not absorb the dye (unstained) were counted as viable, and equations 2 and 3 were used to compute the numbers of viable and non-viable cells.

$$\text{Viable cells (\%)} = \frac{\text{Number of viable cells}}{\text{Total number of cells}} \text{x } 100 \qquad \text{Eq. 2}$$

$$\text{Non-viable cells (\%)} = \frac{\text{Number of non-viable cells}}{\text{Total number of cells}} \text{x } 100 \qquad \text{Eq. 3}$$

Caspase-3/7 activity assay: The enzymatic activities of caspases-3 and -7, important participants in apoptosis, were evaluated using caspase activity assays (Huang *et al.,* 2025). The induction of apoptosis in MCF-7 cells after treatment with the extracts was evaluated by measuring caspase-3/7 activity. After seeding at a density of 1×10^4 in 96-well plates at 37°C, cells were exposed to 50 µg/mL of each extract with the addition of amygdalin in both concentrations of 50 µg/mL and 0.5 mg/mL as positive controls for 24 hours. Following treatment, cells were separated in accordance with the manufacturer's instructions using the enzyme-containing buffer included with the Caspase-3/7 Assay Kit (Sigma-Aldrich, Ac-DEVD-AMC, USA). In each well of a white or opaque 96-well plate, equal amounts (100 µL) of cell lysate and Caspase-3/7 reagent were combined. The plate was incubated for one hour at room temperature in darkness, to allow full caspase-substrate interaction, after being gently shaken to ensure a uniform mixture. A microplate luminometer (CLIA, Yantai, China) was used to measure the luminescence, which was proportional to caspase-3/7 activity, offering further details about the apoptotic pathways that the extracts activated. The obtained luminescence values were expressed as a percentage, and compared with the untreated control group. Additionally, the relative increase in caspase-3/7 activity was used as evidence of apoptosis, caused by the various extracts and was calculated using Equation 4.

$$\text{Caspase} - \frac{3}{7}\text{Activity (\%)} = \frac{\text{Luminescence}_{\text{sample}} - \text{Luminescence}_{\text{blank}}}{\text{Luminescence}_{\text{positive sample}} - \text{Luminescence}_{\text{blank}}} \text{x } 100 \qquad \text{Eq. 4}$$

Lactate dehydrogenase release assay: This experiment, which was performed using the lactate dehydrogenase (LDH) release-cytotoxicity colorimetric kit (Sigma-Aldrich, TOX7, USA), measures the quantity of LDH that leaked into the culture media. When the integrity of the cell membrane is compromised, the intracellular enzyme LDH may react with the extracellular environment (Kadi *et al.,* 2025). The experimental cell samples were aspirated out of the media and centrifuged for five minutes at 4,000 rpm.

At a density of 1×10^4 cells per well, MCF-7 cells were seeded into 96-well plates and left to adhere overnight. The cells were exposed to various extracts at progressively higher concentrations (10 - 100 µg/mL) for 24 hours. Following the treatment period, a fresh 96-well flat-bottom plate was carefully filled with 50 µL of culture supernatant from each well. Each well was then filled with 50 µL of the LDH reaction mixture from the commercial LDH cytotoxicity assay kit. After gently mixing, the plates were incubated for half an hour at 25°C in darkness. A microplate reader was used to detect the optical density at 490 nm. By lysing cells with 1% Triton X-100, a maximal LDH release control was added to ascertain the degree of cell membrane damage (Olofinsan *et al.,* 2025a). Each treatment's cytotoxicity in percentage has been calculated when compared to the maximum release, Equation 5.

$$\text{LDH release (\%)} = \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{low control}}}{\text{Abs}_{\text{high control}} - \text{Abs}_{\text{low control}}} \text{x } 100 \qquad \text{Eq. 5}$$

where: $\text{Abs}_{\text{low control}}$ is Absorbance for untreated cells and $\text{Abs}_{\text{high control}}$ is Absorbance from Triton X-100–treated cells

Bradford protein assay: This investigation aimed to standardize downstream assays, after treatment with plant extracts, and to measure total protein in MCF-7 cells as an indirect indicator of metabolic activity and vitality. After treatment with 50 µg/mL of each plant extract, and amygdalin (at concentrations of 50 µg/mL and 0.5 mg/mL) as positive controls for 24 hours, the cells were rinsed repeatedly with cold PBS, and lysed in ice-cold Radioimmunoprecipitation (RIPA) buffer enriched with a protease enzyme cocktail. After centrifugation for 15 minutes at 4ºC at 10,000 rpm, the supernatants were collected for protein analysis using the BCA assay, following the manufacturer's instructions. Then, 50 µL of BCA Reagent B was combined with 50 µL of BCA Reagent A. Three replicates of each sample and standard, totaling 25 µL each, were used in the 96-well plate (Ahmed *et al.,* 2021). 200 µL of diluted Coomassie Brilliant Blue reagent (Sigma-Aldrich, B6916, USA) was used with a 96-well containing 10 µL of each supernatant for the Bradford assay. After carefully mixing the plate, it was incubated for 30 minutes at 37°C and 5% CO_2. A microplate reader was used to detect absorbance, at 595 nm against a standard curve generated with Bovine Serum Albumin (BSA), from which sample protein concentrations were calculated and expressed in µg/µL (Dastjerdi *et al.,* 2013).

Nitric oxide production assay: The Griess reagent, a measure of oxidative and pro-inflammatory reactions, was used to measure the amount of nitrite ($NO2^-$) in the culture supernatant in order to effectively evaluate the production of nitric oxide (NO). In 96-well microtiter plates, MCF-7 cells were seeded in 96-well plates under conventional growth conditions, injected at a density of 2×10^4 cells per well, and the cells were left overnight and were treated with

plant extracts for 24 hours at doses of 25 and 50 µg/mL. After the incubation period, 100 µL of the culture supernatant was meticulously gathered from every well and combined with 100 µL of newly made Griess reagent (Sigma Aldrich, Germany) in a different 96-well plate. An equal volume (1:1) mixture of 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride and 1% sulfanilamide, both made in 2.5% (v/v) phosphoric acid. In order to allow color formation, the mixture was incubated for 15 minutes at room temperature in the dark. The absorbance was detected with a microplate reader at 550 nm (Mfotie *et al.,* 2018). Results were detected by comparing absorbance measurements to a standard curve produced using multiple dilutions of sodium nitrite. Every test was carried out three times, while results were expressed as µM.

Reactive oxygen species assay: The 2′,7′-dichlorofluorescein diacetate (DCFH-DA) test was used to measure the generated intracellular ROS levels after treatments. MCF-7 cells were seeded at a density of 1×10^4 cells per well in 96-well black-walled plates, and they were left for the whole night. Select plant extracts were added to cells the next day at a dosage of 50 µg/mL. As a positive control, amygdalin has been used at two concentrations: 50 µg/mL and 0.5 mg/mL. Following a 24-hour treatment period, cells were washed off with PBS and incubated for 30 minutes at 37°C in the darkness with a (10 µM) DCFH-DA kit (35845, Sigma-Aldrich) (Olofinsan *et al.,* 2025b). Excessive dye was removed after incubation for 90 minutes, and any remaining dye was gently rinsed out of the cells away from light using PBS. A microplate reader was used to measure the fluorescence intensity, which corresponds to intracellular ROS levels, at an excitation wavelength of 485 nm and an emission wavelength of 535 nm (Olofinsan *et al.,* 2025b). Results were presented as a percentage of relative ROS generation after fluorescence data was adjusted to the untreated control group. Equation 6 was used to determine each treatment group's relative ROS level. Every experiment was carried out in triplicate, and the mean ± standard deviation was used to express the results.

$$\text{Relative ROS (\%)} = \frac{F_{\text{sample}}}{F_{control}} \text{x } 100 \qquad \text{Eq. 6}$$

Adenosine triphosphate assay: As directed by the kit manufacturer (CS224 series, Luminescent, Sigma, USA), it was used to measure the intracellular ATP levels, a sign of metabolically active cells. The cells were resuspended in 50 µL of fresh culture medium after being treated for 24 hours with various plant extracts (50 µg/mL) and the positive control, amygdalin (50 µg/mL and 0.5 mg/mL). 50 µL of ATP reagent and 50 µL of 1X PBS were then added to each well to maintain buffer conditions and guarantee homogeneity. In order to promote cell lysis and maintain the visible signal, the resultant 150 µL reaction mixture was well combined and then incubated for 30 minutes at 25°C in the darkness by wrapping it in foil paper to prevent fluorescence loss upon exposure to light on a plate shaker (SK-L330-Pro, Beijing, China). Following incubation, the entire volume was moved into a 96-well opaque white plate, and a microplate reader was used to measure the luminescence intensity (Olofinsan *et al.,* 2025a). The number of alive cells and the amount of ATP present were directly correlated with the luminous signal. The living cells' ATP luminescence was then measured and expressed as a percentage in relation to the untreated control group (100 %), using Equation 7.

$$\text{ATP (\%)} = \frac{RUL_{\text{sample}}}{RUL_{control}} \text{x } 100 \qquad \text{Eq. 7}$$

Where: Relative light unit (RLU) for sample and untreated cells.

Statistical analysis

The values from each group were compared using a one-way analysis of variance (ANOVA) by Tukey's Honest Significant Difference (HSD) post-hoc test. SPSS version 22 (Chicago, IL, USA) was used to analyze the data for the triplicate determinations. GraphPad Prism (V8.1) was used to create the graphs. Values were expressed as mean ± standard deviation, and each treatment was carried out in triplicate. A statistically significant p-value fell between 0.05 and 0.001.

Results and Discussion

Cell viability: The anti-proliferative properties of some plant extracts, including green tea (G-tea), pomegranate peel (Pom), ginger (Gin), garlic (Gar), and turmeric (Tur), at a concentration of 50 µg/mL, were evaluated in comparison to commercial amygdalin at 50 µg/mL and 0.5 mg/mL. Using the MTT assay, all extracts and controls were examined against the MCF-7 cells. All treatments presented a significant decrease in cell viability when compared with the 100% untreated control. The most noticeable cytotoxic effect of the plant extracts was shown by garlic extract, which decreased MCF-7 viability to 40.63%, Fig. 3. Ginger and turmeric extracts came in second and third value (44.85 % and 47.58%), respectively. Green tea extract and pomegranate peel extract had moderate impacts, lowering viability to 65.11% and 59.47%, respectively. As a positive control, amygdalin had a dose-dependent anti-proliferative action, substantially decreasing viability to 31.11% at 0.5 mg/mL and to 58.02% at 50 µg/mL. Furthermore, cell viability in normal cells (non-cancerous control) remained high, indicating preferential cytotoxicity towards cancer cells at the studied dosages (95.1 % and 88.25%, respectively). These results showed that some of the plant extracts, especially garlic, had cytotoxic effects comparable to or even exceeding those of amygdalin at lower doses, suggesting they may be used as natural anti-cancer agents. The different phytochemical contents of each extract and their distinct actions in regulating cell proliferation are likely responsible for the observed variations in activity. Results were in agreement with (Ghazanfari *et al.,* 2011), who studied the positive effects of garlic extract cytotoxicity on nonmalignant and malignant cells. The findings verified the finding that the quantity and location of methoxy groups

affect cytotoxic action, which was previously demonstrated in asymmetrical analogs of curcumin and 4-amino chalcone compounds (Hariyanti *et al.,* 2022). In MCF-7 cells that overexpress the Human Epidermal growth factor Receptor 2 (HER-2), curcumin has also been demonstrated to decrease HER-2 expression, the capacity to cause apoptosis in a variety of cancer cells, and prevent cell growth (Moga *et al.,* 2021). Comparably, (Esmatabadi *et al.,* 2017), reported that microRNA-21 (Mir-21) prevents dendrosomal curcumin's (DNC) multiple anti-cancer actions in breast cancer cells. Their results indicated that treating drug-resistant breast cancer cells by combining DNC therapy with Mir-21 downregulation may be an effective strategy. The combination of ginger/turmeric extract (20v:80v) had a stronger cytotoxic effect and reduced the cytotoxic effect on T47D breast cancer cell lines (Haryanti *et al.,* 2025). Pomegranate can minimize the U87 brain cancer cells invasion and migration (Zraikat *et al.,* 2024). The study was linked with (Hamdulay *et al.,* 2025), who highlighted how Pom extract positively affected the cell viability, which is essential for creating less harmful cancer treatment options. Safari *et al.,* (2019), reported green tea as a potent natural product with anti-tumor activities on lung carcinoma (A549), prostate (PC3), and breast (MCF-7) cancer cells. The effectiveness of amygdalin as a cancer treatment is still an issue of intense debate in the scientific medical community (Al-Ansari *et al.,* 2024), while the current investigation reported the higher effects on cell viability by garlic, turmeric, and ginger consumption against the amygdalin at the same concentration.

Viable and non-viable carcinogenic cells: The Trypan Blue exclusion assay was applied to assess the anti-proliferative effects of amygdalin and specific plant extracts on MCF-7 cells. With a high vitality of 97.35%, the untreated control MCF-7 cells demonstrated typical growth circumstances. Cytotoxicity values varied when the plant extracts were administered at 50 µg/mL. The extract from garlic rhizome caused the most decrease in cell viability, 43.78 %, resulting in 56.22% non-viable carcinogenic cells, Fig. 4(a and b). The extracts from turmeric and ginger caused 47.12% and 41.88% non-viable cells, respectively. Green tea and pomegranate peel extracts showed moderate anti-proliferative effects, with 68.21% and 66.70% of viable cells, respectively. Amygdalin, the positive control, showed a concentration-dependent impact. Amygdalin lowered cell viability to 62.22% at 50 µg/mL and significantly to 27.45% at 0.5 mg/mL, showing 72.55% non-viable cells. Significantly, of the plant extracts examined, the higher quantity of amygdalin showed a stronger anti-proliferative impact. Selective cytotoxicity toward cancer cells and not healthy counterparts was confirmed by the high vitality of normal cells treated with the same amounts of extracts and amygdalin (93.54% and 95.32% for 50 µg/mL and 0.5 mg/mL, respectively). However, being less successful than high-dose amygdalin in lowering MCF-7 cell viability, these findings indicated that the studied plant extracts, in particular those of garlic, turmeric, and ginger, have strong anti-proliferative potentials. Proteins and other cell components may leak into the extracellular media when a cell dies, which is why they are used as a biomarker for cell death. In addition to the actual cytoplasmic content, dead cells let external substances such as Trypan Blue into their interior and interact with cellular macromolecules since they are unable to preserve the integrity of their membranes (Chan *et al.,* 2020). It's significant to note that alkaloids and their derivatives from plants have a well-established role in cancer treatments (Olofinsan *et al.,* 2023). Different amounts of alkaloid content were found in the phytochemical profiles of the selected plant extracts. The presence of methylxanthine alkaloids, such as caffeine, theobromine, and theophylline, which are well-known for their bioactive qualities, such as antioxidant and anti-proliferative actions, made green tea extract the most abundant source of alkaloids among them (Safari *et al.,* 2019). The phytochemical richness of pomegranate peel extract was more closely linked to polyphenols such as ellagic acid and tannins, but it also showed a moderate amount of alkaloid compounds, including several isoquinoline-type alkaloids (Zraikat *et al.,* 2024; Hamdulay *et al.,* 2025). In contrast, the alkaloid content of turmeric, ginger, and garlic extracts is insignificant (Roy *et al.,* 2020). Curcuminoids in turmeric, phenolic ketones such as gingerol and shogaol in ginger, and organosulfur compounds such as allicin in garlic were instead given responsibility for their biological action. These results suggested that other bioactive components dominated the anti-cancer effects, even if alkaloids might have contributed to the pharmacological potential of some extracts (Esmatabadi *et al.,* 2017).

Apoptosis: A class of proteolytic enzymes known as caspases (cysteinylaspartases) is essential for triggering apoptosis. They are categorized as executioner caspases (−3, −6, −7) and starter caspases (−2, −8, −9). -10). Both intrinsic and extrinsic mechanisms can trigger initiator caspases, which in turn can trigger executioner caspases and induce apoptosis. A crucial indicator for identifying apoptosis is caspase-3/7 activation (Kabir *et al.,* 2017). Apoptosis is an intricate procedure that involves the mobilization of several molecules and can be categorized into two distinct categories: caspase-dependent or caspase-independent. Caspase-dependent pathways may be further separated into extrinsic or intrinsic pathways, as evaluated by the involvement of caspase-8 and caspase-9, which is crucial for activating further DNA cleavage molecules (Looi *et al.,* 2013). Amygdalin and a few plant extracts were tested for their pro-apoptotic effects on MCF-7 cells by measuring their caspase-3/7 activity. Baseline activity was set at 100% for the untreated group. Increased apoptotic induction was revealed by the considerable increase in caspase-3/7 activity observed in all treatments. When examined at 50 µg/mL, the plant extracts that showed the greatest increase in caspase activity were garlic extract (264.55%), turmeric (238.11%), and ginger (188.06%). Green tea and pomegranate peel extracts also had significant effects, enhancing activity to 161.10% and 177.28%, respectively. Amygdalin, a commercial substance, caused a strong dose-dependent apoptotic reaction. Among all evaluated treatments, caspase activity

peaked at 315.45% at the higher dose of 0.5 mg/mL, indicating the most effective apoptotic induction, whereas activity increased to 200.04% at 50 µg/mL, which portray a dose-dependent trend Fig. 5. However, the same quantities of extracts or amygdalin only slightly increased the caspase activity of normal cells (104.59% and 115.03%, respectively), indicating some selective cytotoxicity toward cancer cells. These results demonstrated that high-dose amygdalin functions as a potent apoptosis inducer in MCF-7 cells and that the studied plant extracts, especially garlic and turmeric, had potent pro-apoptotic actions. Actually, it has been suggested that several human cancers are caused by abnormalities in the apoptotic pathway. Therefore, one of the effective tactics in cancer chemotherapy is the use of anti-cancer drugs that trigger apoptosis (Looi *et al.,* 2013). Sfari *et al.,* (2019) investigated the effects of administering green tea extracts on a variety of human diseases, such as promoting antioxidant activity and activating the detoxification system, changing the cell cycle, inhibiting the receptor protein kinase (RTK) and mitogen-activated protein kinase (MAPK) pathways, and inducing the apoptosis pathway that mediates pro- and anti-apoptotic proteins.

Release of LDH: The reversible conversion of pyruvate to lactate, known as LDH catalysis, restricts the quantity of the former molecule that can enter the citric acid cycle for the production of anaerobic energy. Consequently, LDH guarantees a quick supply of energy required for cancer cells to multiply in hypoxic environments, which are prevalent in the majority of tumor microenvironments (Olofinsan *et al.,* 2025a). The LDH release assay showed that the examined plant extracts and amygdalin had distinct cytotoxic effects on MCF-7 cells, while normal cells showed little to no cytotoxicity. All plant extracts, except for pomegranate (27.68%), turmeric (24.58%), and garlic (22.14%), enhanced LDH release in MCF-7 cells to varied degrees; however, no treatment caused any discernible LDH release in normal cells at low or high concentrations; instead, it remained at 0%, Fig. 6. This showed that, even at the higher doses utilized, there was no cytotoxicity or membrane damage in the non-cancerous cells. Extracts of ginger and green tea released 17.66% and 19.21% LDH, respectively, indicating rather less potential effects. In MCF-7 cells, amygdalin also showed a dose-dependent cytotoxic impact, raising LDH release from 11.45% at low dose to 38.14% at high dose, while normal cells showed no release at any level. These results imply that the investigated substances, especially pomegranate extract and high-dose amygdalin, can specifically cause cytotoxicity in breast cancer cells while sparing healthy breast epithelial cells. Since it reduces the possibility of adverse effects frequently related to non-specific cytotoxic medicines, this selective characteristic is essential for possible therapeutic applications. Differences in redox balance, membrane permeability, and metabolic activity between cancerous and healthy cells may be the cause of the selective cytotoxicity. MCF-7 cells are susceptible to phytochemicals that cause necrosis or apoptosis since they frequently have changed membrane composition and increased baseline oxidative stress (Olofinsan *et al.,* 2025b). The high concentration of bioactive chemicals in pomegranate peel and the other extracts that cause oxidative stress, restrict cell growth, and trigger apoptosis while posing little hazard to healthy cells makes them efficient against MCF-7 breast cancer cells (Roy *et al.,* 2020).

Nitric oxide production: Nitric oxide (NO) production, a pro-inflammatory mediator involved in many physiological processes, is crucial for the body's defense. On the other hand, tissue injury and the activation of pro-inflammatory mediators linked to both acute and chronic inflammation might result from its overproduction (Mfotie *et al.,* 2017). Nitric oxide, a signaling molecule with multiple roles, including involvement in the defense system, viral replication, and regulation of inflammatory reactions, is produced by the nitric oxide synthases (NOS) family. Regarding the latter, the decrease in NO production indicates possible anti-inflammatory effects (García-Romo *et al.,* 2025). Consequently, NO inhibitory natural drugs may be useful in the management of the inflammatory response. Plant extracts such as turmeric, ginger, garlic, pomegranate peel, and green tea at 50 µg/mL, as well as commercial amygdalin at 50 µg/mL and 0.5 mg/mL, were used to assess the production of NO - a measure of oxidative and inflammatory response, in MCF-7 cells. The NO level in the untreated MCF-7 control group was 4.65 µM Fig. 7. With varying degrees of anti-inflammatory or oxidative stress-modulating activity, the plant extracts that significantly reduced NO production results were, in ascending order, pomegranate peel extract (2.54 µM), turmeric (3.17 µM), green tea (3.68 µM), garlic (3.74 µM), and ginger (4.23 µM). It's important to observe that, in comparison to the control, the low dose of amygdalin (50 µg/mL) significantly raised NO levels (4.85 µM), while the high dose (0.5 mg/mL) also showed a significant decrease in NO production (2.94 µM). These findings showed that high-dose amygdalin and Pom extract both successfully inhibited NO generation, which may have contributed to their observed anti-proliferative effects. The measured NO response was particular to the metabolic or stress-related pathways of cancer cells, as the normal (non-cancerous) cells did not produce any NO at all under either dose. These results provide some credence to the theory that various plant extracts have the ability to modestly alter NO and other tumor microenvironment variables, potentially increasing their anti-cancer properties. The control and treated groups' differences in NO production were essentially insignificant.

When exposed to stress or bioactive substances, MCF-7 cancer cells produce more NO because they frequently show increased inducible Nitric Oxide Synthase (iNOS) and altered redox homeostasis (Mfotie *et al.,* 2018). Normal cells, on the other hand, do not create NO in non-inflammatory environments and maintain stricter control over NO synthesis. The study's findings about the lack of NO response in healthy cells provided credence to the idea that NO pathways are activated in cancer by metabolic and inflammatory processes, which further supports the plant extracts' therapeutic selectivity.

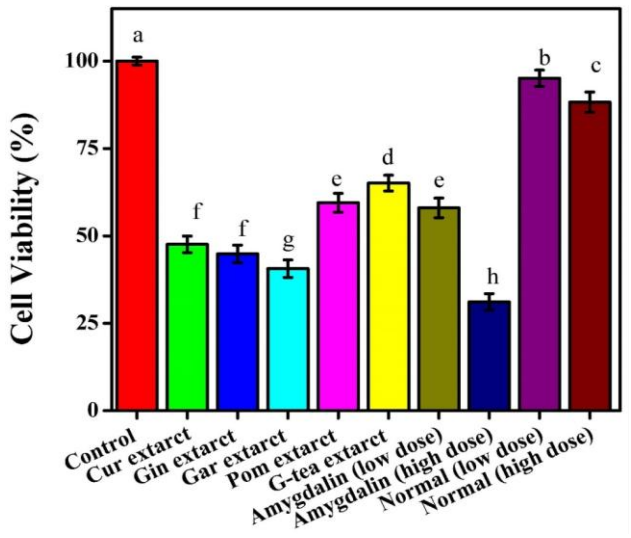

Fig. 3. Effect of various plant extracts and amygdalin on cell viability; Different letters (a, b, c, d, e, f, g, and h) mean significant differences among groups.

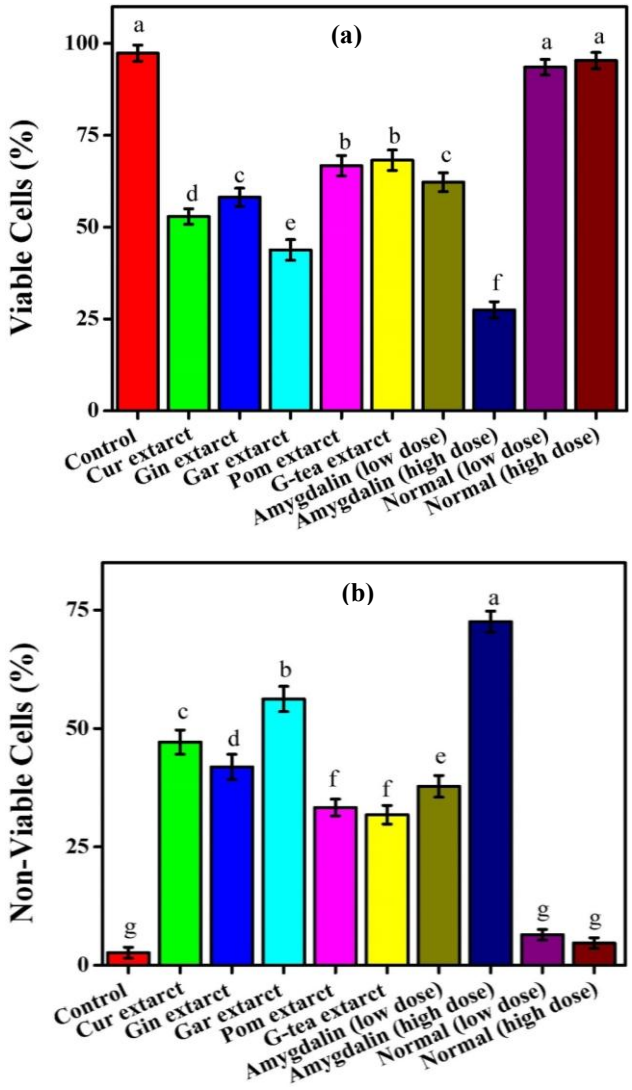

Fig. 4. Effect of various plant extracts and amygdalin on (a) viable cells; (b) non-viable cells; Different letters (a, b, c, d, e, f, and g) mean significant differences among groups

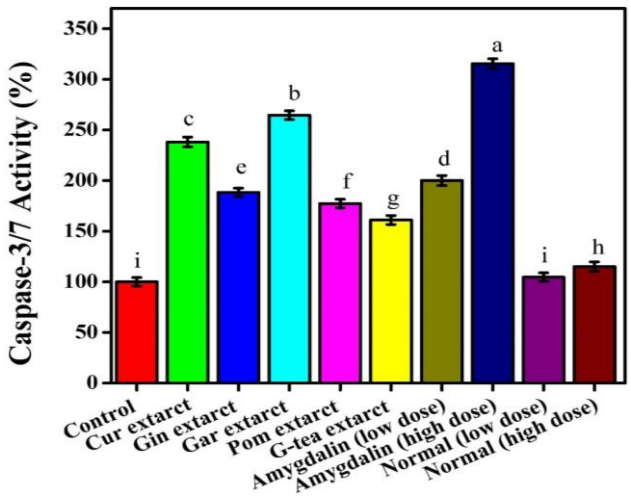

Fig. 5. Effect of various plant extracts and amygdalin on caspase-3/7 activity; Different letters (a, b, c, d, e, f, g, h, and i) mean significant differences among groups.

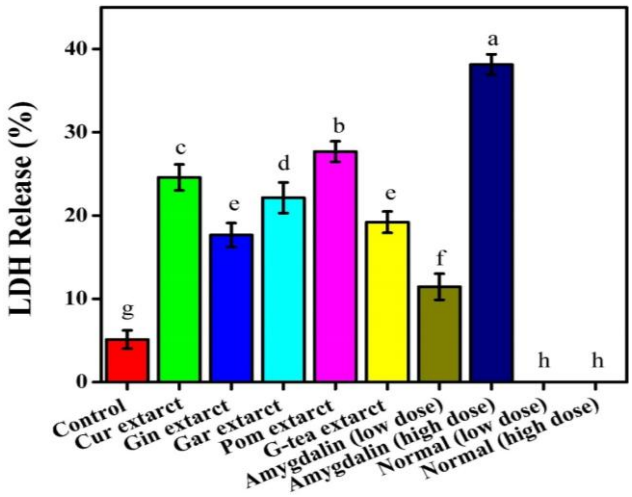

Fig. 6. Effect of various plant extracts and amygdalin on LDH release; Different letters (a, b, c, d, e, f, g, and h) mean significant differences among groups.

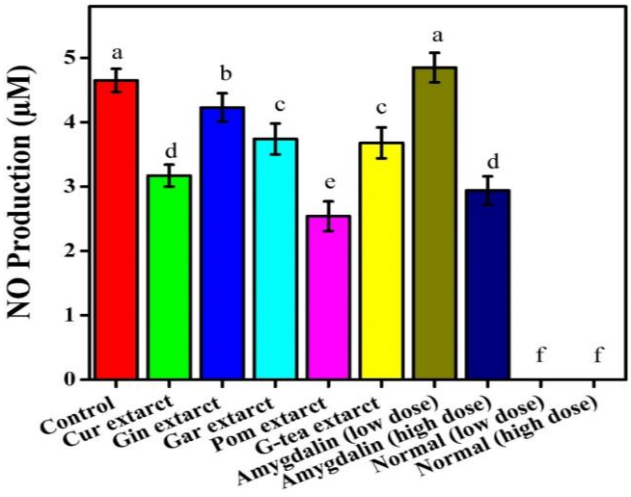

Fig. 7. Effect of various plant extracts and amygdalin on NO production; Different letters (a, b, c, d, e, and f) mean significant differences among groups.

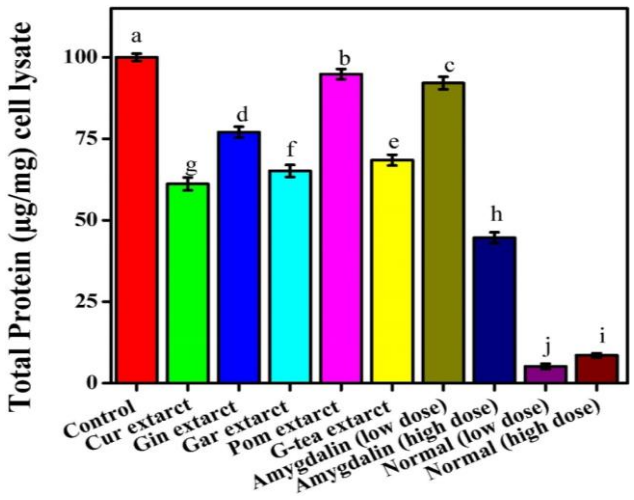

Fig. 8. Effect of various plant extracts and amygdalin on total protein; Different letters (a, b, c, d, e, f, g, h, i, and j) mean significant differences among groups.

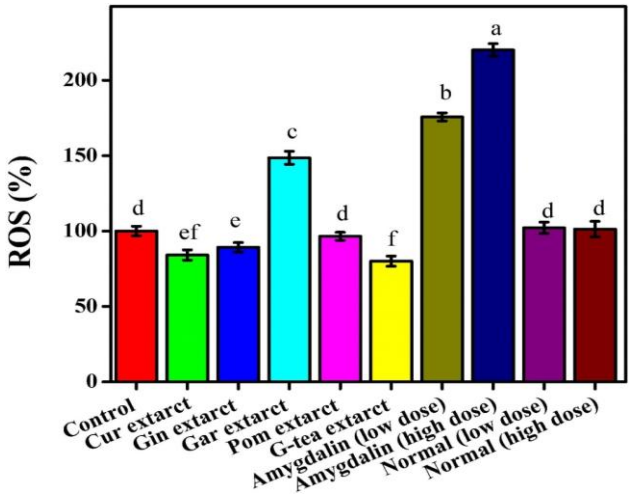

Fig. 9. Effect of various plant extracts and amygdalin on ROS levels; Different letters (a, b, c, d, e, and f) mean significant differences among groups.

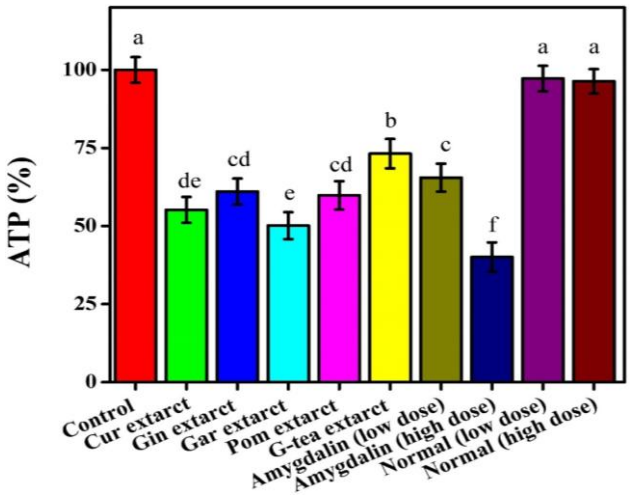

Fig 10. Effect of various plant extracts and amygdalin on ATP levels; Different letters (a, b, c, d, e, and f) mean significant differences among groups

Bradford protein: The amount of cytotoxicity and cellular damage, caused by each substance was indicated by the total protein content in MCF-7 cell lysates, after treatment with the selected plant extracts and amygdalin. All plant extracts at 50 µg/mL decreased total protein levels, indicating a reduction in cell viability and proliferation compared to the untreated control (100 µg/mL). Significant protein decreases (65.11 and 61.14 µg/mg, respectively) were observed in response to garlic and turmeric extracts, indicating effective anti-proliferative action. Additionally, the protein level was decreased by ginger and green tea extracts to 77.02 and 68.45 µg/mg, respectively. Among the plant treatments, pomegranate peel extract had the least impact; its protein level was nearly identical to the control (94.85 µg/mg), suggesting a less significant influence on total cell mass or perhaps a delayed cytotoxic effect. In light of its phytochemicals' time-dependent nature, cytostatic rather than cytolytic mode of action, or antioxidant qualities that may temporarily support cell integrity, the comparatively high protein level observed in MCF-7 cells treated with pomegranate peel extract probably indicates a delayed or mild cytotoxic effect (Zraikat *et al.,* 2024; Hamdulay *et al.,* 2025). A dose-dependent effect was demonstrated by amygdalin; the high dose (0.5 mg/mL) markedly decreased protein content to 44.63 µg/mg, whereas the low dose (50 µg/mL) maintained higher protein levels (92.1 µg/mg), Fig. 8. This suggests that amygdalin exhibits moderate cytotoxicity at lower concentrations and more aggressive cell lysis at higher concentrations. It is important to specify whether protein extraction was carried out on the controls because the protein levels in the "normal" groups (treated non-cancerous cells) were notably recorded as 5.17 and 8.54 µg/mg due to the absence of cell death compared with the cancerous cells. The decrease in total cellular protein indicated that the extracts, especially high-dose amygdalin, turmeric, and garlic, had a major cytotoxic or anti-proliferative effect on MCF-7 cells. It was established that sonicated pomegranate peel extract decreased total protein in MCF-7 cells in a dose-dependent manner. Furthermore, it has been shown that pomegranate extracts may be capable of cell cycle arrest and down-regulation of genes involved in DNA repair (Yapa *et al.,* 2024). In contrast to more directly cytotoxic agents such as garlic or turmeric, which contain compounds (allicin, curcumin) known to interfere with proteostasis, mitochondrial function, and signaling pathways for nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mechanistic target of Rapamycin (mTOR) that regulate protein synthesis and degradation, the comparatively mild effect of pomegranate peel may be due to its slower modulation of cellular pathways (Roy *et al.,* 2020). This supports the contention that protein synthesis and cellular homeostasis are more violently disrupted by elevated amygdalin levels (Christodoulou *et al.,* 2022). Interestingly, research indicates that amygdalin may contribute to cytotoxicity by inhibiting protein turnover and downregulating proteasome activity in breast cancer cells (Al-Ansari *et al.,* 2024).

Reactive oxygen species: ROS can be classified as reactive oxygen species, including reactive anions with oxygen atoms such as peroxides and oxygen ions, as well as free radicals. They may result from tissue-specific enzymes,

aerobic respiration, or the metabolism of xenobiotic substances by microsomal cytochrome P450. Oxidative stress - caused by an excess of ROS, can damage DNA, alter the dynamics of the actin cytoskeleton, and decrease the integrity of the plasma membrane (Looi *et al.,* 2013). ROS is generated, particularly when cells experience environmental or chemical stresses, and may be a contributing factor to apoptosis or cell cycle arrest (Olofinsan *et al.,* 2025a). Treatment with specific plant extracts and amygdalin had significant effects on the levels of ROS in MCF-7. Turmeric and ginger extracts significantly decreased ROS generation to 84.11% and 89.25%, respectively, in comparison to the control group (100%), indicating a minor antioxidant impact or modification of oxidative stress pathways. On the other hand, garlic extract significantly raised ROS levels to 148.63 %, suggesting the formation of oxidative stress, which may be correlated to the cytotoxicity and death of cancer cells. Green tea extract significantly decreased ROS levels to 80.05%, exhibiting a strong antioxidant effect in accordance with its polyphenolic contents, while pomegranate peel extract slightly increased ROS (96.54%), Fig. 9. With low and high dosages increasing ROS levels to 175.68% and 220.15%, respectively, amygdalin demonstrated a dose-dependent increase in ROS, highlighting its pro-oxidant function in triggering oxidative stress and possible death for MCF-7 cells. The control and normal groups' ROS levels were almost the same (100%, 102.18%, and 101.27%) since neither group received any treatment, preserving the breast cells' natural oxidative balanced state, respectively. These results were in line with earlier research showing that substances derived from plants can function as pro-oxidants or antioxidants, affecting ROS-mediated signaling pathways to either stop the growth of cancer cells or cause their death. The study was in agreement with (Haryanti *et al.,* 2017), who documented that proper combination of turmeric and ginger, suppressed the synergistic interaction between ROS generation and cancer cell proliferation. Redox equilibrium - the control of important transcription factors, and the preservation of numerous vital physiological processes all depend on maintaining low to moderate levels of reactive nitrogen species (RNS) and ROS (Moga *et al.,* 2021; Jomova *et al.,* 2023). Extracts from ginger, green tea, and turmeric dramatically lowered ROS levels in MCF-7 cells. Curcuminoids, which scavenge free radicals and strengthen cellular antioxidant defenses, are probably the reason turmeric reduced ROS (Esmatabadi *et al.,* 2017). Due to its high catechin content, which is known to neutralize free radicals and shield cells from oxidative stress, green tea had a comparable impact (Khan *et al.,* 2023; Korkunc *et al.,* 2025). ROS levels were slightly reduced by ginger extract, possibly as a result of gingerols that regulate oxidative stress pathways and support redox equilibrium (Esmatabadi *et al.,* 2017). Pathological circumstances and exposure to stressful environmental variables led to the excessive production of ROS. These adverse effects usually include DNA damage, protein changes, lipid peroxidation, and ion channel opening (Christodoulou *et al.,* 2022).

Intracellular ATP: Eukaryotic cells contain mitochondria, a special organelle that produces ATP, the energy source that enables cell survival in an infected host (Olofinsan *et al.,* 2025b). When compared with the 100% untreated control, the ATP assay results established that all tested plant extracts at 50 µg/mL, significantly decreased the ATP levels in MCF-7 breast cancer cells. The extracts of garlic and turmeric showed the highest inhibitory effects, lowering ATP content to 50.12% and 55.17%, respectively. This indicates a significant reduction in metabolic activity and cellular viability. Green tea extract exhibited a significantly moderate effect, reducing ATP to 73.18%, although extracts from ginger and pomegranate peels also demonstrated significant decreases, with ATP levels of 61.03% and 59.83%, respectively. Dose-dependent cytotoxicity was demonstrated by the amygdalin. Also, the ATP levels decreased to 65.49% at a low dose and more sharply to 40.07% at a high dose. Significantly, both low and high doses of amygdalin-treated normal cells, maintained their ATP levels near the control (97.22% and 96.35%), signifying that the drug is selectively cytotoxic to cancer cells Fig. 10. The findings indicated that amygdalin and the selected plant extracts significantly reduced the energy metabolism and viability of MCF-7 cells, hence establishing their possible anti-proliferative qualities. Similar studies on water extract from *Solanum nigrum* showed that treatment caused significant ATP depletion in MCF-7 cells over time, which was linked to cytotoxicity and viability loss (Khan *et al.,* 2023). However, several extracts (such as pomegranate and green tea) caused less severe ATP decreases, which was consistent with findings of antioxidant-rich extracts that preserve mitochondrial function while having moderate cytostatic effects compared to fast cytotoxicity. According to reports, green tea catechins may exhibit two different effects: pro-oxidant or pro-apoptotic effects at greater doses and antioxidant benefits at lower concentrations that protect mitochondrial integrity (Li *et al.,* 2016). The trend of a slight effect is observed in proliferation assays, despite the fact that results from direct ATP assays with green tea in MCF-7 cells are less frequently observed. Amygdalin's results support the concept that many anticancer drugs exhibit dose dependence, with low to moderate doses affecting ATP levels to some extent, and larger doses causing a major energy crisis consistent with the induction of necrosis or apoptosis. This was reflected in the literature, where substances that enhance ROS or target the machinery involved in mitochondrial ATP synthesis only cause abrupt decreases in ATP once threshold concentrations were reached (Lee *et al.,* 2016). Significant changes in intracellular ROS and ATP levels coincided with the decrease in MCF-7 cell viability after treatment with garlic and turmeric extracts, indicating that their antiproliferative activity was influenced by modulation of oxidative stress and cellular energy metabolism. Apoptosis and decreased cellular proliferation are characterized by compromised mitochondrial activity,

which is indicated by the decrease in ATP generation. Moreover, the induction of planned cell death rather than nonspecific cytotoxicity is supported by the simultaneous activation of caspase-3/7 and decrease in cell viability. Together, these results show that the studied extracts inhibit the development of breast cancer cells by coordinating effects on apoptotic signals, mitochondrial bioenergetics, and oxidative balance.

Conclusion

Breast cancer is still a serious global health concern, compounds originating from plants are being researched as potential anticancer treatments. Bioactive substances produced from plants are being investigated more and more as safer and more efficient substitutes for traditional cancer treatments, which frequently have serious side effects and lower quality of life. This study showed that some selected plant extracts, particularly those of garlic, turmeric, and ginger, have potent and specific anti-proliferative effects on MCF-7 breast cancer cells via a wide range of mechanisms, such as apoptosis induction, ROS generation, ATP depletion, and membrane damage. Some of these effects were on par with or better than those of high-dose amygdalin. Extracts from green tea and pomegranates exhibited fewer cytotoxic effects on normal cells, but they were still very active. More research into the processes and therapeutic uses of these plant-based chemicals is necessary, in accordance with these findings, which also suggest their potential use as natural, selective anti-cancer medicines.

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