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Research Paper

Anthocyanin Extraction and Purification from Red Onion Peels (Allium cepa) and Its Applications Against the CAL-51 Cell Line

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ABSTRACT

Background: Red onion (Allium cepa L.) peels represent an abundant agricultural byproduct rich in bioactive flavonoids, yet their therapeutic potential against aggressive breast cancer models remains poorly characterized. This study aimed to extract and purify anthocyanins from red onion peels, characterize their active constituents, and evaluate their selective in vitro cytotoxicity against the triple-negative breast cancer (TNBC) cell line CAL-51 relative to non-tumorigenic Normal Human Fibroblast (NHF) cells.

Methods: Anthocyanins were extracted with acidified aqueous ethanol, purified using silica gel G60 column chromatography and characterized by reversed-phase high-performance liquid chromatography (RP-HPLC). Cytotoxicity was evaluated through 72-hour MTT assays on CAL-51 and NHF cells, confirmed by phase-contrast morphological analysis and analyzed using one-way ANOVA.

Results: Through RP-HPLC, malvidin and peonidin were characterized as the major monomeric anthocyanidins. According to the results, the purified extract demonstrated a potent and cell line-dependent dose effect for the cytotoxicity of CAL-51 cells with IC50 = 247.5 µg/mL (maximum inhibition: 63.73% at 1000 µg/mL), accompanied by morphological hallmarks of apoptosis. In contrast, the extract exhibited much lower toxicity against NHF cells (IC50=1767 µg/mL; maximum inhibition: 17.39% at 10,000 µg/mL), resulting in a good selectivity index of 7.1.

Conclusion: Purified red onion peel anthocyanins were powerful, selective anti-proliferative and pro-apoptotic agents against TNBC in vitro without harming normal cells. This sets a path to exploiting agricultural waste as a source of potential natural therapeutic agents for the individualised treatment of breast cancer in an economically viable manner.

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Introduction

Anthocyanins are obtained from plants which relate in the flavonoid group. They give many fruits, vegetables, and flowers their red, violet, and blue colors. These substances' potential health-promoting qualities, such as their antimicrobial, hepatoprotective, cardioprotective, antioxidant, and anticancer effects, have drawn a lot of attention [1–3].

Extracting and purifying anthocyanins from vegetable waste is crucial for their potential use in biological applications. Extraction methods and purification techniques (e.g., chromatographic methods) impact yield and chemical stability (which influences availability) [4]. Anthocyanins have been shown in cancer research to inhibit cell division, trigger apoptosis, and alter pathways that regulate, such as NF-κB, in vivo or in vitro situations [5].

Compelling evidence for anthocyanins' anticancer potential encourages investigation of their impact on cell lines derived from breast cancer, despite the restriction of direct investigations using CAL-51 cell lines. The CAL-51 cell line, a model of human breast cancer recognized for its triple-negative phenotype, is used in this investigation to assess pure anthocyanins' bioactivity. Among them ostsevere and resistant forms of breast cancer are represented by this classification. Originally extracted from a patient's pleura who had breast adenocarcinoma, CA L-51 cells have a nearly normal karyotype (46, XX), making them an uncommon but useful model for researching tumorigenic mechanisms that happen without significant chromosomal abnormalities [6].

The choice of triple-negative (ER-, PR- and HER2) CAL-51 cells provides a severe model without common treatment receptors [7]. Furthermore, CAL-51 exhibits near-diploid karyotype stability (46, XX), facilitating the analysis of drug-response phenotypes [8]. PI3K/Akt and MAPK/ERK signalling pathways have been frequently associated with oncogenic signalling in TNBC; CAL-51 has shown sensitivity to combinations against these pathways (e.g. PI3K+IGF1R; FGFR inhibition), supporting them as molecular indicators [9]. Testing pure red-onion anthocyanins in CAL-51 may show supplemental or alternative anticancer potential, given increasing proof that anthocyanins have pro-apoptotic and antiproliferative effects in breast cancer models and that onion peel anthocyanins are bioactive [10].

The main goals of this study are to: (1) devise an effective, environmentally safe technique for the extraction and purification of peels from red onion anthocyanins; and (2) examine the purified anthocyanin fraction's bioactivity against the cell line for human breast cancer CAL-51. By conducting this research, we hope to provide new opportunities for using agricultural waste as a source of valuable bioactive substances and possibly aid in the hunt for natural cancer treatment supplements.

Materials and Methods

Plant Material and Extraction of Anthocyanins

Red onion (*Allium cepa* L.) bulbs were procured from a local commercial supplier in Baghdad, Iraq, and washed several times with deionised water to remove surface contaminants. Approximately 80.0 g of the prepared red onion peel material was homogenised, crushed into a fine powder, and macerated in 150 mL of an aqueous ethanol solvent system (70:30, v/v) to maximise anthocyanin recovery [11]. The sample was subsequently incubated at 4°C for 24 h under complete exclusion of light.

Following incubation, the coarse plant debris was removed by initial filtration through multi-layered fabric gauze. The resulting filtrate was centrifuged at 1,000 × *g* for 10 min at 4°C to sediment any remaining particulate matter. To render the extract suitable for downstream cell culture applications. The organic solvent component was removed under reduced pressure using a rotary evaporator maintained at 55°C under vacuum until complete evaporation of ethanol was achieved [11].

Column Chromatographic Purification

Partial purification of the crude anthocyanin extract was performed using open-column chromatography packed with silica gel G60 as the stationary phase. Elution was carried out using methanol as the mobile phase, and fractions were collected sequentially based on their differential affinity to the stationary phase and their elution behaviour. To confirm the presence of phenolic hydroxyl groups indicative of anthocyanin structures [12].

High-Performance Liquid Chromatography Analysis

Qualitative identification and characterisation of the purified anthocyanin constituents were performed using a SYKAM high-performance liquid chromatography system (SYKAM GmbH, Munich, Germany) [13]. Chromatographic separation was conducted using a reversed-phase C18-ODS column (250 mm × 4.6 mm i.d., 5 µm particle size) at ambient

temperature. Detection was performed using a UV-Vis detector set to an analytical wavelength of 520 nm, where the anthocyanin chromophore exhibits its peak absorbance in the visible spectrum. The isocratic elution was performed at a constant flow rate of 0.8 mL/min. The mobile phase consisted of 2% (v/v) formic acid in water, with the pH adjusted to 7.0 [13] and methanol (95:5, v/v). Identification of specific anthocyanin species, namely malvidin and peonidin, was based on the retention times (t_r) of sample peaks under identical chromatographic conditions to pure external reference standards (5 ppm).

Cell Lines and Culture Conditions

In this study, we evaluated the cytotoxic activity and selective potential of a purified anthocyanin extract using two distinct human cell lines. The CAL-51 human breast cancer cell line, derived from the malignant pleural effusion of a female patient with metastatic breast cancer [8], was used as the tumorigenic model. This cell line exhibits a triple-negative phenotype (ER^-, PR^-, $HER2^-$), has a near-diploid karyotype (46, XX), and is considered a clinically relevant model for studying drug responses in triple-negative breast cancer (TNBC). As a non-tumorigenic control, the Normal Human Fibroblast (NHF) cell line [14] was used to screen the safety and selectivity of the intervention.

All cell lines were routinely cultured in Minimum Essential Medium (MEM; US Biological, USA) supplemented with 10% (v/v) heat-inactivated foetal bovine serum (FBS; Ebsdorfergrund, Germany), 100 IU/mL of penicillin and 100 µg/mL streptomycin (Capricorn-Scientific, Germany) [15]. The cultures were maintained at 37°C under a humidified atmosphere of 5% CO_2. All experiments were carried out using logarithmic (exponential) growth phase cells (optimized metabolic activity, steady-state proliferation rates and experimental reproducibility).

In Vitro Cytotoxicity Assay (MTT Assay)

Cell viability and treatment-induced cytotoxicity were quantitatively assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay [16, 17]. was performed to quantify cell viability and cell death induced by anticancer treatments. This assay is based on the succinate dehydrogenase enzyme activity present in metabolically active cells, which converts yellow (water-soluble) tetrazolium salt (MTT) into purple (insoluble) formazan crystals. The weight of formazan formed is a direct function of the number of living cells and can therefore be quantified as an indicator of cell viability.

Briefly, CAL-51 and NHF cells were implanted in sterile 96-well flat-bottomed microplates (NEST Biotech Co., Wuxi, China) at a density of 1×10^4 cells per well (100 µL complete culture medium). The plates were incubated at 37°C in a humidified 5% CO_2 incubator for 72 h to promote cell adhesion and establish the monolayer equilibrium of each plate. After the stabilization phase, old spent medium was aspirated and replaced with fresh medium supplemented with increasing concentration of purified anthocyanin extract. In the case of CAL-51 cells, we used a two-fold dilution series from 31.2 to 1000 µg/mL (specifically: 31.2, 62.5, 125, 250, 500 and 1000 µg/mL). A wider concentration range (312 to 10,000 µg/mL) was tested for NHF cells to adequately establish the safety margin and selectivity index.

Subsequently, each well received 28 µL of MTT dye solution (2 mg/mL diluted in PBS; Elabscience Biotechnology Co., Wuhan, China) followed by 3 h incubation at 37°C to enable the formation of formazan crystals after returning the plates to the incubator post-exposure for a total lasting treatment exposure time of 72 h. Next, the culture supernatant was carefully removed, and 100 µL of dimethyl sulphoxide (DMSO) was added to each well to dissolve insoluble formazan crystals. The plates were shaken on an orbital shaker for 15 min at room temperature to allow crystals to be completely solubilised. Using a microplate spectrophotometer, we measured the optical density (OD) of each well at 492 nm. The percentage of cell cytotoxicity was calculated using the following formula:

$$Cytotoxicity\ (\%) = [(OD\_Control - OD\_Sample) / OD\_Control] \times 100$$

where OD_Control is the average absorbance of the untreated control wells (carrying cells and culture medium but no test compound), and OD_Sample indicates the absorbance of all treated wells. Half-maximal inhibitory concentrations (IC_{50}), which represent the amount of the test compound necessary to inhibit 50% cell viability as compared with untreated controls, were obtained by non-linear regression fitting of the dose-response curves [16].

Statistical Analysis

Data are shown as the mean ± standard deviation (SD), derived from independent triplicate experiments (n = 3). The data underwent statistical evaluation using GraphPad Prism software (version 8.0; GraphPad Software). To evaluate various treatment groups, a one-way ANOVA was performed, followed by Tukey's HSD post-hoc test for multiple pairwise comparisons.

Statistical significance was established at $p < 0.05$.

Results

The application of HPLC analyze the component in this well-use analytical technique, extract could partly separate, identify, and characterized monomeric anthocyanins composing the complex phytochemical matrix of red onion peel extracts. Selected chromatographic conditions exhibited superb resolution, peak symmetry, and reproducible retention features, which confirmed that the analytical method well-matched the objectives for qualitative anthocyanin profiling.

Table 1 provides a summary of the chromatographic retention times and peak characteristics of separated components. The analytical profile of the red onion peel extract showed five large, well-resolved peaks with high signal-to-noise ratios eluting at t_r 3.77, 4.05, 5.87, 7.99 and 8.21 min (Figure 1). The good peak resolution and signal purity obtained for all chromatographic peaks are indicative of the interaction between anthocyanins with the C_{18} stationary phase under isocratic conditions during the analyses made in this work. Correspondingly, the peaks eluting at medium retention times (i.e. peaks 3 to 4 and peak 5) are identified as anthocyanin species creating stronger hydrophobic associations with the reversed-phase packing material.

Table 1. Chromatographic parameters and peak identification of anthocyanins from red onion (*Allium cepa* L.) peels.

Peak No.	Retention Time (t_r, min)	Identification Method	Assigned Compound
1	3.77	External standard t_r matching	Malvidin
2	4.05	UV-Vis spectral profiling	Anthocyanin derivative
3	5.87	UV-Vis spectral profiling	Anthocyanin derivative
4	7.99	External standard t_r matching	Peonidin
5	8.21	UV-Vis spectral profiling	Anthocyanin derivative

External reference standards of malvidin and peonidin (5 ppm each) were similarly analysed under identical chromatographic parameters to allow for unambiguous qualitative identification of the major anthocyanin species. This resulted in a sharp, symmetrical peak for malvidin at a retention time of 3.77 min (Figure 2). A comparison of this characteristic profile to the extract chromatogram proved that the peak eluting at t_r = 3.77 min in sample one corresponds directly to malvidin. Likewise, the peonidin reference

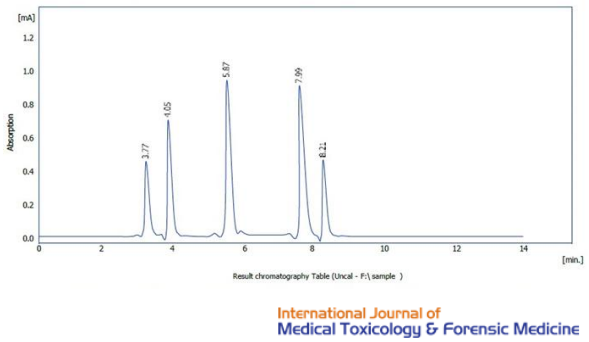

Figure 1. HPLC chromatogram showing the separation and

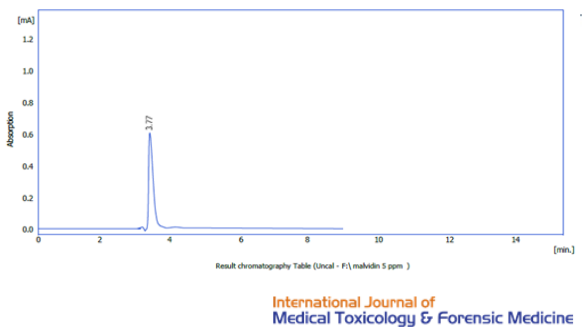

Figure 2. HPLC chromatographic profile of the substance with recognized peak for malvidin.

standard presented a peak of very distinct and preferably defined from those at 7.90 min (Figure 3). The retention behaviour of this standard at t_r = 7.99 min matches the peak shown in the sample profile, confirming that peonidin is present in the red onion peel extract after purification. The small retention time difference (0.09 min) also remained within the acceptable analytical tolerance of retention-time-based

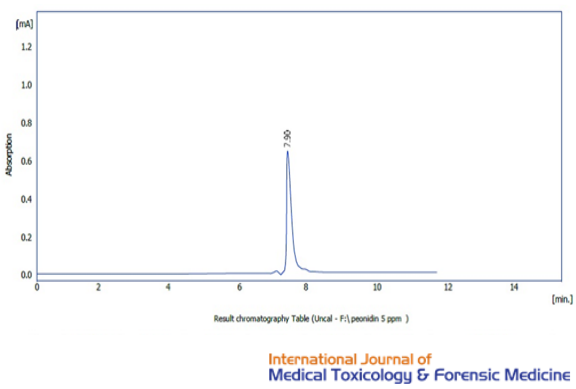

Figure 3. HPLC chromatographic profile of the substance with recognized peak for peonidin.

identification and is due to expected inter-run variability.

The cytotoxic effects of the purified red onion peel anthocyanin extract were evaluated against the human triple-negative breast cancer cell line CAL-51 and the non-tumorigenic Normal Human Fibroblast (NHF) control line using the MTT colorimetric cell viability assay. The percentage of cell growth inhibition across a range of treatment concentrations following 72 h of continuous exposure is presented in Table 2.

threshold intracellular concentration of the bioactive species is necessary before significant anti-proliferative or pro-apoptotic cascades are initiated. On the contrary, the extract barely exerted any toxicity on normal NHF fibroblasts even at very high concentrations. In NHF cells, at the maximum concentration of 10,000 µg/mL, cytotoxicity did not exceed 17.39% with a gradual decline towards near-background levels at lower

Table 2. In vitro cytotoxicity (%) of purified red onion peel anthocyanins against CAL-51 breast cancer cells and Normal Human Fibroblasts (NHF) following 72 h of treatment.

CAL-51 Concentration (µg/mL)	Cytotoxicity (%)	NHF Concentration (µg/mL)	Cytotoxicity (%)
Control (0.0)	0.00	Control (0.0)	0.00
31.2	1.19	312	0.16
62.5	10.79	625	1.84
125	17.14	1250	7.21
250	31.14	2500	10.05
500	54.75	5000	15.78
1000	63.73	10,000	17.39

No significant difference in cytotoxicity was observed among groups at the lowest concentrations tested for both cell lines, specifically at 31.2 µg/mL for CAL-51 and 312 µg/mL for NHF. In contrast, there is a significant increase in cytotoxicity values at higher treatment concentrations, demonstrating a clear concentration-dependent inhibitory pattern Table 2.

Anthocyanin extract produced high-dose-dependent cytotoxic activity in CAL-51 breast cancer cell line, with 63.73% as the maximum growth inhibition observed at 1000 µg/mL in comparison to control (Figure 4). A similar inhibition effect continued without interruption at middle doses, with cytotoxicity of 54.75% and 31.14% for 500 and 250 µg/mL. The cytotoxic response was considerably reduced by a factor of three to seven at concentrations 31.2–125 µg/mL (10–50 µM anthocyanins), suggesting that the

concentrations (Table 2). This minimal effect on non-target cells suggests a favorable safety profile.

Figure 5 shows that the selectivity for pure anthocyanin extract is further shown by the half-maximal inhibitory concentration (IC_{50}) values that were derived from the non-linear regression analysis of the dose-response curves. The IC_{50} value of the CAL-51 triple-negative breast cancer line was 247.5 µg/mL, indicating that only a small amount of the extract (low cell viability after exposure) is needed for a 50% decrease in the number of viable cancer cells. Interestingly, the NHF normal fibroblast line, on the other hand, had a very high IC_{50} value of 1767 µg/mL. This relevant difference produces a selectivity index (SI) close to 7.1, showing that the extract is seven times more toxic towards malignant cells than towards healthy human fibroblasts. For closely related strains

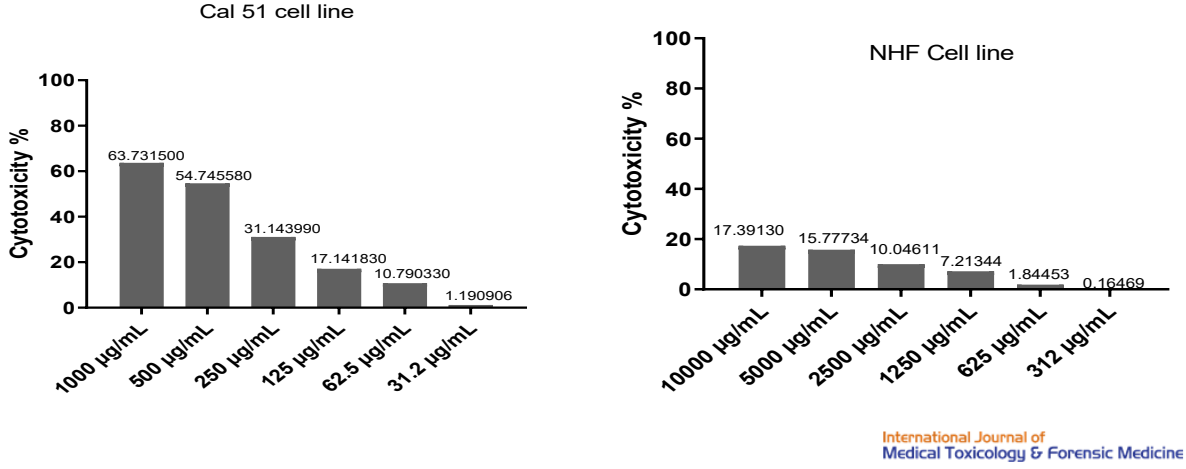

Figure 4. Cytotoxicity (%) of the tested compound against the **Cal 51 and NHF cell lines** at different concentrations.

such as these, a large differential in the respective IC_{50} values highlights the remarkable therapeutic selectivity for red onion peel anthocyanin extract.

At the end of the 72-h treatment period, qualitative evaluation of cell viability and structural integrity was conducted through phase-contrast imaging of treated and untreated cell monolayers (Figure 6). Morphological results largely validated the quantitative data from the MTT colorimetric assay.

features of apoptosis and marked cytotoxic stress (H50-cell death score between 2 and 3). Such alterations are: cell shrinkage, rounding, membrane blebbing, nuclear condensation and high detachment from the substrate due to events of apoptotic cell death.

The morphology of untreated controls showed healthy, elongated, spindle-shaped fibroblastic morphology with intact cell membranes and parallel alignment in the NHF cell line. Compared to untreated

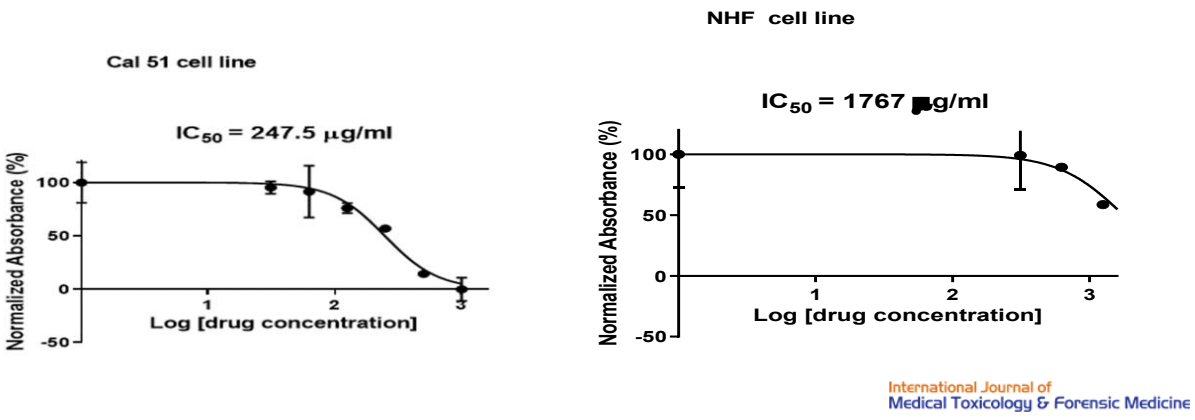

Figure 5. Dose-response curve of the tested drug on Cal 51 and NHF cell lines, showing normalised absorbance (%) versus log-transformed drug concentration.

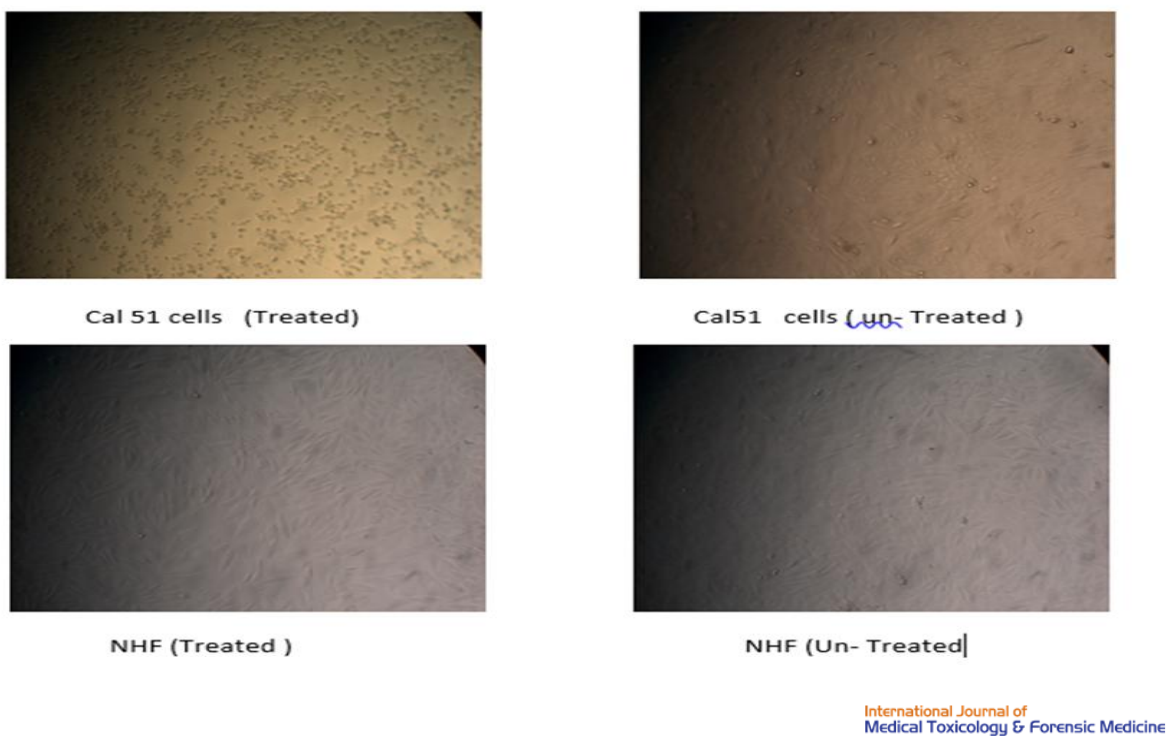

Figure 6. Evaluating Treated and Untreated Cal 51 and NHF Cell Lines Under a Microscope.

Untreated CAL-51 breast cancer cells showed typical malignant features of epithelial morphology: a confluent, cohesive monolayer with polygonal shapes and a high degree of adherence to the culture surface (Fig. By contrast, CCAL-51 cells exposed to the purified anthocyanin extract developed classic morphologic

NHF cells, NHF cells treated with the anthocyanin extract at equivalent concentrations retained their characteristic spindle-like morphology and structural integrity with only minor changes. In a comparative analysis of the structural degeneration in normal cells and breast cancer cells, we showed clear visual evidence

that red onion anthocyanin had selective cytotoxic effects on breast cancer cells, as evidenced by the lack of severe morphological damage to normal cells relative to extensive structural degradation seen in the breast cancer cells.

In order to conclusively establish treatment-related effects statistically, OD raw values acquired from the microplate reader were analyzed through one-way-ANOVA. The average OD values for the treated groups and their level of significance against the untreated controls are shown in Figure 7.

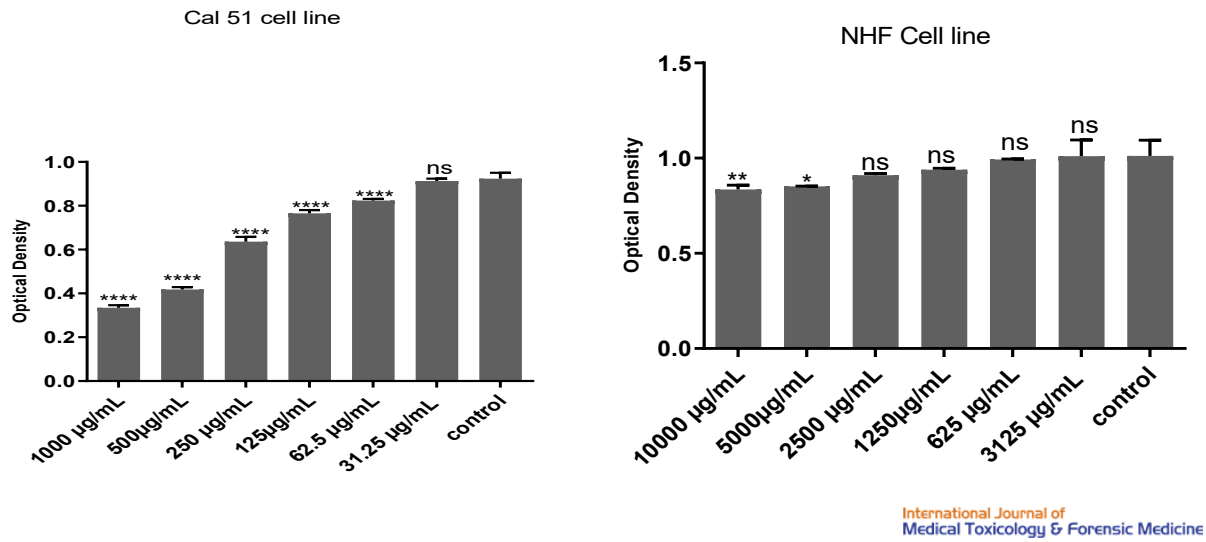

Figure 7. The investigated compound has a different effect on the viability of normal and cancerous cell lines.

The results are depicted in Figure 7. For the NHF cell line, no significant difference in OD between the groups was observed at both low and medium concentrations 312-2500 mu g/mL ($p > 0.05$, indicated as "ns"). On the other hand, there is a significant reduction in OD value for CAL-51 at 62.5-1000 µg/mL ($p < 0.0001$).

A statistically significant decrease in cell viability in the normal NHF fibroblast was detected only upon treatment at the two highest concentrations: 5000 µg/mL ($p < 0.05$) and 10,000 µg/mL ($p < 0.01$). This shows minimal cytotoxicity to normal human fibroblasts in physiological and therapeutic concentration ranges of the anthocyanin extract. In contrast, the CAL-51 breast cancer line is extremely sensitive to virtually all tested doses, with profound, statistically significant decreases in viability. Apart from the lowest concentration of 31.25 µg/mL ($p > 0.05$), all other concentrations of treatment (62.5 to 1000 µg/mL) led to a highly significant decrease in optical density when compared to the untreated control group ($p < 0.0001$). This quantitative statistical analysis provides further evidence for the conclusion that purified anthocyanin extract has a very strong and selective anti-proliferative activity against breast cancer cells with a very good safety profile in normal human tissues.

Discussion

The HPLC analysis of the purified extract derived from red onion (*Allium cepa* L.) peels revealed a well-resolved chromatographic profile characterised by five distinct peaks. Through comparison of retention times with high-purity external reference standards, two principal monomeric anthocyanidins were unambiguously identified: malvidin ($t_R = 3.77$ min) and peonidin ($t_R = 7.99$ min). The identification of these specific anthocyanins is highly consistent with previous phytochemical investigations of red onion varieties. Fossen and Andersen[18] isolated peonidin and malvidin analogues from red onion skins, confirming the structural diversity of B-ring substitution patterns in *Allium cepa* tissues. Similarly, Samota et al [19] reported that red onion peels harbour a complex mixture of anthocyanin glycosides beyond the commonly reported cyanidin derivatives, supporting the chromatographic profile observed in the present study. The sharp peak symmetry, adequate signal-to-noise ratios, and reproducible retention characteristics observed herein confirm the robustness and chromatographic efficiency of the reversed-phase C_{18} isocratic elution system employed.

The purified anthocyanin extract demonstrated a pronounced, dose-dependent cytotoxic effect against the CAL-51 human breast cancer cell line, achieving a maximum growth inhibition of approximately 60% at a concentration of 1000 µg/mL. This concentration-dependent modulation of cancer cell viability is in agreement with a substantial body of literature demonstrating that plant-derived anthocyanins and their aglycones effectively suppress the proliferation of

aggressive breast cancer models. Furthermore, Aqil et al [20] showed that bilberry-derived anthocyanidins (malvidin and peonidin) effectively inhibited the growth of TNBC cells in vitro and in vivo, which was correlated with the suppression of NF-κB signalling. Regulatory Effects of Fruits and Fruit Extracts on Cancer Pathways. The half-maximal inhibitory concentration (IC50) observed in the present study is similar to those of other anthocyanin-rich extracts tested against TNBC cell lines, as reviewed by Sood et al [21]. It has been recorded that malvidin and its glycosides are capable of inducing apoptosis, causing cell cycle arrest, and inhibiting cell proliferation through the modulation of various signalling pathways.

At high concentrations, the extract produced considerable cytotoxicity, which can be mechanistically attributed to the capacity of anthocyanins to induce intrinsic (mitochondrial) apoptosis. Lin et al [22] In a comprehensive review of the mechanisms by which anthocyanins induce apoptosis in cancer cells, it has been shown that these compounds can promote the expression of Bax and downregulate Bcl-2, culminating in an increased ratio of Bax/Bcl-2 towards the promotion of apoptosis. This change results in the disruption of the outer mitochondrial membrane, releasing cytochrome c into the cytosol, which activates a sequential cascade (the caspase cascade), inducing programmed cell death [22, 23]. similarly reported that lower anthocyanin concentrations typically confer anti-inflammatory or antioxidant benefits without significantly inducing cell death, a phenomenon consistent with the moderate cytotoxicity observed at intermediate concentrations in the present study. This threshold-dependent behaviour aligns with pharmacological findings indicating that many flavonoids require high intracellular concentrations to dramatically reduce cancer cell survival [24].

The cytotoxicity profile of the purified anthocyanin extract exhibited a striking and statistically significant divergence between the CAL-51 breast cancer cell line and the NHF normal human fibroblast line. Whilst the extract demonstrated significant dose-dependent cytotoxicity in CAL-51 cells, even at the maximum concentration tested (10,000 µg/mL), the extract exhibited remarkably low cytotoxicity against NHF cells, with inhibition not exceeding 16%. This selective cytotoxicity is in strong agreement with earlier investigations demonstrating that cancer cells are inherently more susceptible to anthocyanin-induced oxidative stress, mitochondrial disruption, and apoptosis compared with their non-tumorigenic counterparts [25, 26]. In non-cancerous cells, anthocyanins function as potent antioxidants and radical scavengers, upregulating phase II cytoprotective enzymes via the Keap1-Nrf2-ARE pathway, thereby conferring protection against oxidative damage [27, 28]. Giampieri et al [28] whilst Palungwachira et al [27] confirmed the antioxidant properties of anthocyanins in primary dermal fibroblasts. Conversely, in malignant microenvironments where the antioxidant buffering capacity is already compromised, anthocyanins at higher concentrations act as selective pro-oxidants, triggering intracellular ROS accumulation through mitochondrial disruption or copper ion mobilisation, which preferentially drives cancer cells towards apoptosis [29].

Additionally, recent systematic reviews and meta-analyses have revealed that anthocyanins inhibit essential survival pathways in triple-negative breast cancer models. Through meta-analysis, Rabelo et al [30] also showed that anthocyanins downregulate the Akt/mTOR pathway, induce activation of caspase-3, caspase-8 and cleaved PARP in TNBC cells, as well as inhibit invasion and migration of cells.CAL-51 is a clinically relevant model for such drug responses as the cell line is triple negative (ER^-, PR^-, $HER2^-$), and is near normal (46, XX) karyotypically [31]. The altered redox balance, dysregulated cell cycle control, and higher metabolic demands inherent to CAL-51 cells make them particularly susceptible to bioactive compounds that disrupt mitochondrial function, induce oxidative stress, or trigger apoptotic cascades.[31,32]. The significant loss in viability observed in the CAL-51 cell line, particularly with plant-derived polyphenolic anticancer agents, is consistent with the growing body of evidence supporting the therapeutic potential of dietary anthocyanins against TNBC [20, 30, 33].

The purified A. cepa anthocyanin extract's high selectivity index has significant clinical significance because conventional chemotherapeutic agents lack specificity [20]. The ability of red onion peel anthocyanins to selectively target CAL-51 cells whilst sparing normal fibroblasts highlights their potential as safe, natural therapeutic agents or as adjuvants in combination therapies. Aqil et al [20] demonstrated that combining non-toxic dietary anthocyanidins with paclitaxel reduced the IC_{50} of the chemotherapeutic agent by approximately 20-fold in drug-resistant TNBC models, mediated through the downregulation of ATP-binding cassette (ABC) transporters and the suppression of NF-κB signalling. Since red onion skins are a plentiful, inexpensive farming waste material, their use as a source of highly bioactive anthocyanins is in accordance with green chemistry and sustainable pharmacology concepts [34].

Conclusion

This study demonstrates that purified red onion peel anthocyanins (mainly malvidin and peonidin) exhibited a strong and selective in vitro cytotoxic effect against triple-negative breast cancer cells without affecting normal fibroblast cells. Instead of being just another dose-dependent, natural extract, these data demonstrate the therapeutic potential for converting plentiful agricultural waste into cost-effective, less toxic and highly focused oncological interventions. This selective cytotoxicity, probably related to different redox responses and apoptosis, definitely provides a sound pharmacological basis for the use of Allium cepa waste as a sustainable chemopreventive or synergistic adjuvant in standard BC therapies, with an interest in further in vivo potency and mechanistic studies.

Ethical Approval

The Central Biological Ethics Committee of the College of Medicine, Babylon, approved the experimental procedures (no. 1248/14-11-2025).

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Conflicts of Interest

The authors report there are no competing interests to declare.

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