nutraceuticals

MDPI

Article

Effects of *Nephelium lappaceum* L. Peel Extracts on Cancerous Cells

Paola K. Mireles-Elizalde [1], Margarita L. Martinez-Fierro [2], Mauricio A. Salinas-Santander [1], Ana C. Cepeda-Nieto [1], Idalia Garza-Veloz [2], Juan Ascacio-Valdés [3], Raúl Rodríguez-Herrera [3], Sunday Sedodo-Nupo [4] and Jesús A. Morlett-Chávez [1,5,*]

1 Health Research Department, Faculty of Medicine Saltillo Unit, Autonomous University of Coahuila, No. 935 Boulevard Venustiano Carranza Street, Saltillo 25000, CU, Mexico; krinahp.pm@gmail.com (P.K.M.-E.); mauriciosalinas@uadec.edu.mx (M.A.S.-S.); anacepeda@uadec.edu.mx (A.C.C.-N.)
2 Molecular Medicine Laboratory, Academic Unit of Human Medicine and Health Sciences, Autonomous University of Zacatecas, Km. 6 Zacatecas-Guadalajara Road, Zacatecas 98160, ZA, Mexico; margaritamf@uaz.edu.mx (M.L.M.-F.); idaliagv@uaz.edu.mx (I.G.-V.)
3 Food Research Department, School of Chemistry, Autonomous University of Coahuila, No. 935 Boulevard Venustiano Carranza Street, Saltillo 25280, CU, Mexico; alberto_ascaciovaldes@uadec.edu.mx (J.A.-V.); raul.rodriguez@uadec.edu.mx (R.R.-H.)
4 University Hospital, Autonomous University of Coahuila, No. 1291 Calzada Francisco I. Madero Street, Saltillo 25000, CU, Mexico; sundaynupo@yahoo.com
5 Clinical Laboratory Department, General Hospital No. 2, Mexican Institute of Social Security, Saltillo 25017, CU, Mexico
* Correspondence: antoniomorlett@uadec.edu.mx

Abstract

Natural polyphenols have gained increasing attention as potential anticancer agents, although the selective effects of many plant-derived extracts on cervical cancer cells remain insufficiently characterized. Rambutan (*Nephelium lappaceum*) peel, an underutilized agro-industrial by-product enriched in ellagitannins and related polyphenols, represents a promising source of bioactive compounds for nutraceutical and cervical cancer research. The objective of this research was to investigate the effect of rambutan peel polyphenolic extract (RPPE) on cervical cancer cells (HeLa) and non-malignant cells. Cell viability, morphological alterations, and transcriptional responses associated with apoptosis, survival, and cell-cycle regulation were examined. RPPE selectively reduced the viability of HeLa cancer cells while exerting limited effects on human dermal fibroblasts (HDFa). Treated HeLa cells exhibited morphological and transcriptional changes consistent with apoptosis and growth inhibition. However, minimal responses were observed in HDF treated with RPPE. Overall, these findings provide evidence that RPPE differentially affects malignant and non-malignant cells, supporting further investigation of rambutan peel-derived polyphenols as sustainable bioactive compounds for cervical cancer research and future nutraceutical applications.

Keywords: cervical cancer cells; natural polyphenolic extract; rambutan peel extract; selective cytotoxicity

Check for updates

Academic Editor: Shih-Yi Huang

Received: 10 January 2026
Revised: 7 July 2026
Accepted: 27 July 2026
Published: 27 August 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

Cervical cancer (CC) remains one of the most common malignancies affecting women worldwide and continues to represent a major public health burden, particularly in low- and middle-income countries [1–4]. Persistent infection with high-risk human papillomavirus

(HPV), especially types 16 and 18, is the principal etiological factor associated with cervical carcinogenesis through the action of the viral oncoproteins E6 and E7, which disrupt key cellular regulatory pathways [3,4]. Current therapeutic strategies for CC include surgery, radiotherapy, and chemotherapy; however, treatment outcomes may be compromised by adverse effects, drug resistance, tumor recurrence, and reduced quality of life [2,3,5,6]. Consequently, there is growing interest in identifying bioactive compounds that selectively target cancer cells while minimizing toxicity to normal tissues [7–11]. Natural products have long served as therapeutic agents, and plant-derived polyphenols have attracted particular attention because of their ability to modulate multiple molecular pathways involved in cancer progression [2,4]. These compounds have been reported to inhibit proliferation, induce apoptosis, suppress angiogenesis, and interfere with metastatic processes in several cancer models [2,4,7–11].

Biocompounds with multitargeted mechanisms and generally favorable safety profiles have promoted their investigation as potential complementary agents in cancer prevention and treatment. Rambutan (*Nephelium lappaceum* L.) is a tropical fruit from the Sapindaceae family that is widely cultivated in Southeast Asia and southern Mexico [12–14]. Although the edible pulp is commercially valued, the peel represents a major agro-industrial by-product that is often discarded despite being rich in bioactive phytochemicals [12,13,15]. Previous phytochemical studies have demonstrated that rambutan peel contains high concentrations of hydrolyzable tannins and ellagitannins, including geraniin, corilagin, ellagic acid, and ellagic acid derivatives, which are considered the principal contributors to its biological activity [11,14,16,17]. These compounds have been associated with antioxidant, antimicrobial, antiviral, anti-inflammatory, and anticancer properties, making rambutan peel an attractive source of value-added nutraceutical ingredients [11,14,18,19].

Several studies have reported the anticancer potential of rambutan-derived extracts. In human osteosarcoma cells, rambutan extract induced apoptosis, promoted G2/M cell-cycle arrest, increased caspase-3 and caspase-9 expression, and reduced Bcl-2 and Erk1/2 levels [16,20]. Similarly, methanolic extracts from rambutan tissues enhanced apoptosis in HepG2 hepatocellular carcinoma cells [14]. Rambutan peel extracts have also demonstrated cytotoxic activity against both cancerous and non-cancerous cell lines, suggesting the presence of biologically active compounds that modulate cellular survival pathways [17,21]. Although tannins may exhibit antinutritional properties at high concentrations, these compounds are largely responsible for the beneficial biological activities attributed to rambutan peel [12,16,17].

Despite the growing evidence supporting the pharmacological potential of rambutan-derived polyphenols, information regarding their effects on cervical cancer cells remains limited. In particular, the molecular responses associated with apoptosis, survival signaling, and cell-cycle regulation in HeLa cells have not been fully characterized. Therefore, this study aimed to evaluate the cytotoxic and molecular effects of rambutan peel polyphenolic extract (RPPE) on HeLa cervical cancer cells and normal human dermal fibroblasts (HDFa), with emphasis on cellular viability, morphological alterations, and transcriptional responses associated with apoptosis, survival, and cell-cycle regulation.

2. Materials and Methods

2.1. Ethics Statement

The study was approved by the Research Ethics Committee of the General Hospital of Saltillo under NOM-012-SSA3-2012 (approval No. 23/2024).

2.2. Hybrid Rambutan Peels Polyphenolic Extraction

Rambutan fruits of the Mexican variety used for extract preparation were obtained from the Soconusco region, Chiapas, Mexico. Rambutan peels were separated, dehydrated in a conventional oven (MRC Laboratory Instruments Ltd., Holon, Israel) at 50 °C for 48 h, and processed in a Grindomix GM 300 Knife mill (RETSCH GmbH, Haan, Germany) to obtain a homogeneous powder. The plant material was identified as *N. lappaceum* L. based on its commercial source and morphological characteristics. Polyphenolic extraction was carried out using a hybrid ultrasound/microwave procedure with an Ultrasound/Microwave Cooperative Workstation (Nanjing ATPIO Instruments Manufacture Co., Ltd., Nanjing, China), operating at a microwave frequency of 2450 MHz and an ultrasound frequency of 25 kHz. Water was used as the extraction solvent at a 1:16 m/v ratio (62.5 g of rambutan peel in 1 L of water). Ultrasound-assisted extraction was performed for 20 min, followed by microwave-assisted extraction for 5 min at 70 °C. This short exposure to elevated temperature minimized the degradation of polyphenolic compounds. The resulting extracts were filtered under low-light conditions to protect phenolic constituents and remove residual peel particles. Extracts were then transferred to glass bottles wrapped in aluminum foil and stored at −18 °C until further use. The 1:16–0 extract was selected for subsequent analysis because of its high polyphenol content (307.57 ± 20.27 mg/g dry matter) and the use of water as a safe, non-toxic, and environmentally friendly solvent. The plant material corresponded to the same batch used for the preparation and chemical characterization of the rambutan peel polyphenolic extract (RPPE) [12,13].

2.3. Polyphenol Quantification

Total hydrolyzable polyphenols were quantified using the Folin–Ciocalteu method. Briefly, 400 µL of sample extract was mixed with 400 µL of Folin–Ciocalteu reagent and incubated for 5 min. Subsequently, 400 µL of sodium carbonate and 2.5 mL of distilled water were added. Absorbance was measured at 790 nm using a Biomate 3 spectrophotometer (Thermo Spectronic, Madison, WI, USA). Gallic acid (0–500 ppm) was used as the calibration standard, and results were expressed as mg gallic acid equivalents (GAE)/g dry matter. All measurements were performed in triplicate. Condensed polyphenols were determined using the HCl–butanol assay. Samples (500 µL) were mixed with 3 mL of HCl–butanol solution (1:10, v/v) and 100 µL of ferric reagent (1:9, v/v), followed by incubation at 100 °C for 60 min. After cooling, absorbance was measured at 460 nm. Catechin (0–1000 ppm) was used as the standard, and results were expressed as mg catechin equivalents (CE)/g dry matter. All analyses were conducted in triplicate [12,13].

2.4. Cell Culture

HeLa (ATCC CCL-2) cervical cancer cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin/amphotericin B (all Invitrogen, Carlsbad, CA, USA). The adult human primary dermal fibroblasts (HDFa) (ATCC PCS-201-012) cell line was resuspended in Fibroblast Basal Medium (ATCC, Manassas, VA, USA) containing Fibroblast Growth Kit–Serum-Free and 1% penicillin/streptomycin/amphotericin B (all ATCC, Manassas, VA, USA). Both cell lines were incubated at 37 °C in a humidified atmosphere with 5% CO_2 using a Forma Direct Heat CO_2 Incubator (Thermo Fisher Scientific, Waltham, MA, USA).

2.5. Cell Viability Assay

HeLa and HDFa cells were seeded in 96-well plates at 1.5×10^4 cells/well and incubated for 24 h. RPPE was dissolved in sterile culture medium by sonication for 15 min and added at final concentrations of 50, 100, 150, and 200 µg/mL. Cells were

incubated for 24, 48, or 72 h at 37 °C in 5% CO_2. Untreated cells served as controls. Cell viability was determined using the MTT assay. Briefly, 10 µL of MTT reagent (final concentration 0.5 mg/mL; Sigma-Aldrich, St. Louis, MO, USA) was added to each well and incubated for 4 h. Formazan crystals were dissolved with 50 µL DMSO, and absorbance was measured at 570 nm using a Smart Reader 96 microplate reader (Accuris Instruments, Edison, NJ, USA). All experiments were performed in triplicate following a randomized experimental design [12,16].

2.6. RNA Isolation and cDNA Synthesis

HeLa and HDFa cells (15×10^4 cells per well) were seeded in 24-well plates and cultured for 24 h in a humidified atmosphere with 5% CO_2 at 37 °C. Cells were then treated with 200 µg/mL of RPPE for an additional 24 h. Untreated cells served as controls. After incubation, total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. RNA integrity was verified by 1% agarose gel electrophoresis. RNA concentration and purity were determined by measuring absorbance at 260 nm and calculating the 260/280 ratio, using a NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Samples with a 260/280 ratio greater than 1.60 were used for subsequent analysis. RNA was stored at −80 °C until use.

Complementary DNA (cDNA) was synthesized from total RNA using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystem, Foster City, CA, USA) according to the manufacturer's protocol. Briefly, the reaction mixture contained 2 µL of 10X RT Buffer, 0.8 µL of dNTPs Mix (100 mM), 2 µL of 10X random primers, 1 µL multiScribe™ reverse transcriptase, 1 µg of RNA, and nuclease-free water to a final volume of 20 µL. The thermal cycling conditions were as follows: 25 °C for 10 min, 37 °C for 120 min, 85 °C for 5 min, followed by a final hold at 4 °C. Synthesized cDNA was stored at −20 °C until further use [12,14,20].

2.7. Quantitative Real-Time PCR (qRT-PCR)

The qRT-PCR was performed using a StepOnePlus™ Real-Time PCR System (Applied Biosystem, Foster City, CA, USA) in 96-well PCR plates. Synthesized cDNA (20 ng per reaction) was used as a template in a final volume of 20 µL, containing 1X SYBR Green PCR Master Mix (Applied Biosystem, Foster City, CA, USA) and 500 nM gene-specific primers. The thermal cycling conditions were as follows: initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing at 60 °C for 1 min. All samples were analyzed in duplicate, and a melt curve analysis was performed at the end of each run to verify amplification specificity. Relative mRNA expression levels of *BCL2*, *P53*, *AKT*, *BAX*, *P21*, and *GAPDH* were calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences are shown in Supplementary Table S1.

2.8. Statistical Analysis

All variables were tested in triplicate following a randomized experimental design. Data are presented as the mean ± standard deviation (SD). Statistical differences between control and treated groups were assessed using Student's *t*-test, with statistical significance set at $p < 0.05$. Statistical analyses were performed using SigmaPlot, version 14.5 (Systat Software, San Jose, CA, USA), and IBM SPSS Statistics, version 25.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Rambutan Peel Polyphenolic Extract

The extraction yielded 12.16% (7.6 g from 62.5 g of dried peel). The enriched extract exhibited higher concentrations of hydrolyzable, condensed, and total polyphenols than the crude extract (Table 1). LC–MS/MS analysis identified ellagitannins as the predominant polyphenolic constituents, with geraniin, corilagin, and ellagic acid derivatives among the major compounds detected (Table 2).

Table 1. Quantification of polyphenolic fractions in rambutan peel extracts.

Extract Type	Hydrolyzable Fraction (mg GAE/g DW)	Condensed Fraction (mg CE/g DW)	Total Polyphenols (mg GAE/g DW)
Crude extract	201.60 ± 6.43	141.84 ± 2.26	343.44 ± 6.43
Enriched extract	221.38 ± 3.62	157.10 ± 5.57	378.48 ± 9.19

Table 2. Major phenolic constituents identified in rambutan peel extract by LC–MS/MS analysis.

Peak	tR (min)	Tentative Identification	Classification
P1	25.92	Tergallagic acid hexoside dimer	Ellagitannin
P2	26.21	Corilagin	Ellagitannin
P3	28.12	Geraniin	Ellagitannin
P4	32.13	Ellagic acid pentoside	Ellagitannin derivative
P5	33.71	Ellagic acid	Phenolic acid derivative

3.2. RPPE Reduces Viability in HeLa but Not in Normal Fibroblasts

An initial assessment was conducted to evaluate the cytotoxic effects of RPPE on HeLa cancer cells and to determine its selectivity relative to normal human dermal fibroblasts (HDFa). Both cell lines were treated with increasing concentrations of RPPE (50, 100, 150, and 200 μg/mL) and incubated for 24, 48, and 72 h. Figure 1A shows that cell viability at zero remains close to 100% across all concentrations in HeLa and HDFa cell types, indicating that RPPE does not exert immediate viability effects before incubation. In HeLa cells, treatment at lower concentrations (50 and 100 μg/mL) for 24 h resulted in only minor reductions. However, higher concentrations (150 and 200 μg/mL) reduced cell viability to approximately 60% and 40%, respectively. These effects became more pronounced over time, with HeLa cell viability decreasing to approximately 30% and 20% at higher RPPE concentrations after 48 h, respectively. At 72 h, viability declined to below 20% at both concentrations, confirming a sustained concentration-dependent cytotoxic response. In contrast, HDFa cells exhibited markedly greater resistance to RPPE. As shown in Figure 1B, HDFa viability remained near 100% at all concentrations after 24 h. Treatment with 150 μg/mL resulted in a modest reduction (~80%). After 48 h of treatment, cell viability decreased to approximately 65% at 150 μg/mL and 55% at 200 μg/mL. By 72 h, significant HDFa cytotoxicity was observed only at 200 μg/mL, while viability remained high (~85–90%) at lower concentrations. Collectively, these findings suggest that RPPE exerts selective cytotoxic effects against HeLa cells in a dose- and time-dependent manner while showing lower toxicity toward HDFa cells under the experimental conditions evaluated (Figure 2).

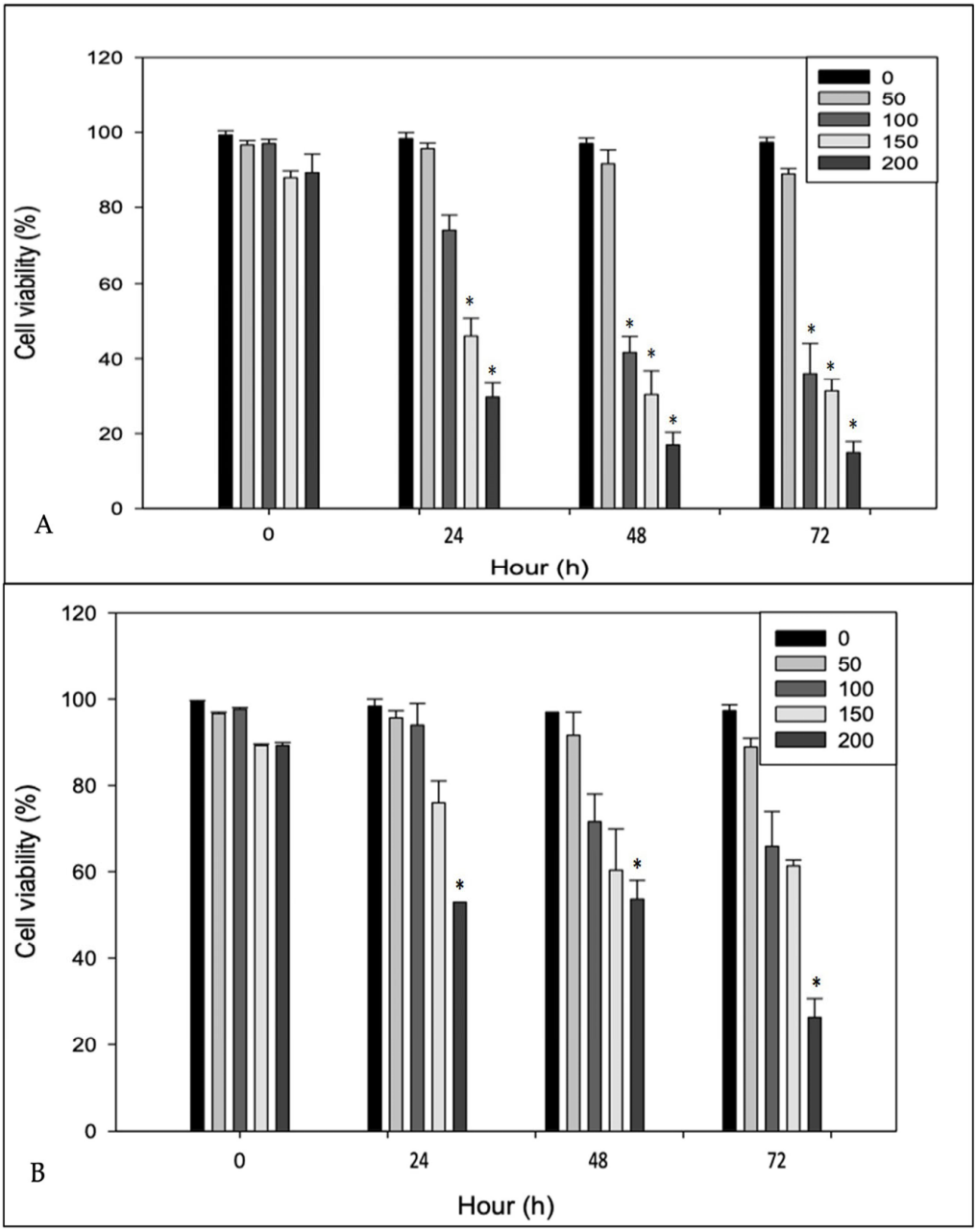

Figure 1. Effect of rambutan peel extract on HeLa cell viability as a function of time and concentration. (**A**)—HeLa; (**B**)—HDFa cells. The graphs show cell viability (%) at 0, 24, 48, and 72 h after exposure to different concentrations of the extract (0, 50, 100, 150, and 200 µg/mL). Cell viability progressively decreased as extract concentration and exposure time increased, suggesting a dose- and time-dependent cytotoxic effect. * Indicates a statistically significant difference ($p < 0.05$).

3.3. Morphological Changes

HeLa cells treated with RPPE for 24 h exhibited concentration-dependent morphological alterations consistent with apoptotic cell death (Figure 3). Untreated control cells (Figure 3A) maintained their typical epithelial-like morphology, characterized by elongated shapes, strong adherence to the culture surface, intact cellular architecture, and minimal cellular debris, indicative of a healthy proliferative state. In contrast, RPPE-treated cells displayed progressive morphological changes, including cell shrinkage, loss of intercellular contacts, reduced adherence, cellular rounding, cytoplasmic condensation, membrane blebbing, and accumulation of cellular debris (Figure 3B–D). These alterations became increasingly evident as RPPE concentrations increased. At 200 µg/mL (Figure 3D), extensive

cell detachment and fragmentation were observed, suggesting severe cellular damage and advanced stages of apoptosis. The marked dissimilarity between untreated and treated cells reflects the progressive loss of cellular integrity induced by RPPE. Overall, these morphological findings are consistent with the reduction in cell viability observed in the MTT assay and support the induction of apoptosis in HeLa cells following RPPE exposure.

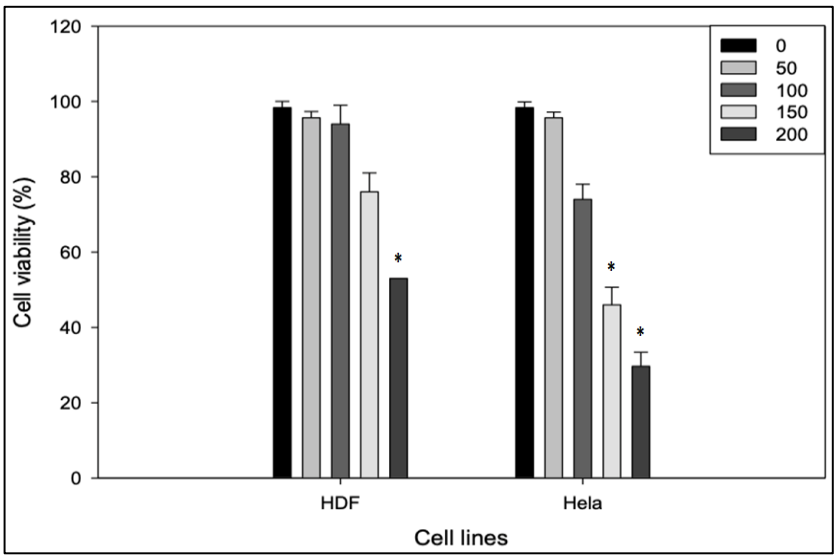

Figure 2. Selective cytotoxic effect of RPPE on HeLa and HDF cell lines. Cell viability of normal human dermal fibroblasts (HDF) and HeLa cervical cancer cells following 24 h of treatment with increasing concentrations of Rambutan Peel Polyphenolic Extract (RPPE; 0, 50, 100, 150, and 200 µg/mL). Viability was assessed using an MTT assay. HeLa cells exhibited a clear dose-dependent decrease in viability, with significant cytotoxicity effects observed at higher concentrations. In contrast, HDF cells maintained relatively high viability, even at 150 and 200 µg/mL, indicating the selective toxicity of RPPE toward cancer cells. Data are presented as mean ± SD from three independent experiments. * Indicates a statistically significant difference ($p < 0.05$).

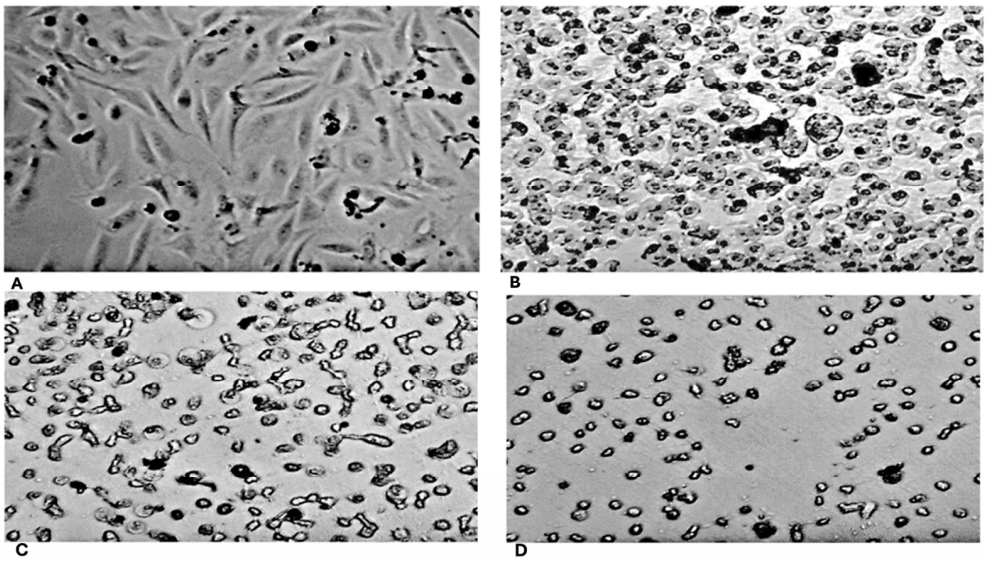

Figure 3. Morphological changes in HeLa cells following RPPE treatment. Phase-contrast microscopy images of HeLa cells after 24 h of treatment with increasing concentrations of RPPE. (**A**)—Untreated control cells exhibit normal epithelial morphology with elongated, adherent cells and intact membranes. With increasing RPPE concentrations, cells progressively lose adherence, become rounded, and show cytoplasmic shrinkage, membrane blebbing, and cellular fragmentation, indicative of apoptotic and necrotic processes. (**B**)—RPPE 100 µg/mL; (**C**)—RPPE 150 µg/mL; (**D**)—RPPE 200 µg/mL.

3.4. Gene Expression

The half-maximal inhibitory concentration (IC_{50}) for HeLa cells was determined at 175 µg/mL, and this concentration was therefore selected for subsequent gene expression analyses. Figure 4 illustrates the relative mRNA expression levels of genes associated with cell survival (*AKT*, *BCL2*), apoptosis (*P53*, *BAX*), and cell-cycle arrest (*P21*) in HeLa cells treated with RPPE, compared to RPPE-treated HDF cells (Figure 4A–C). All expression levels were normalized to *GAPDH*.

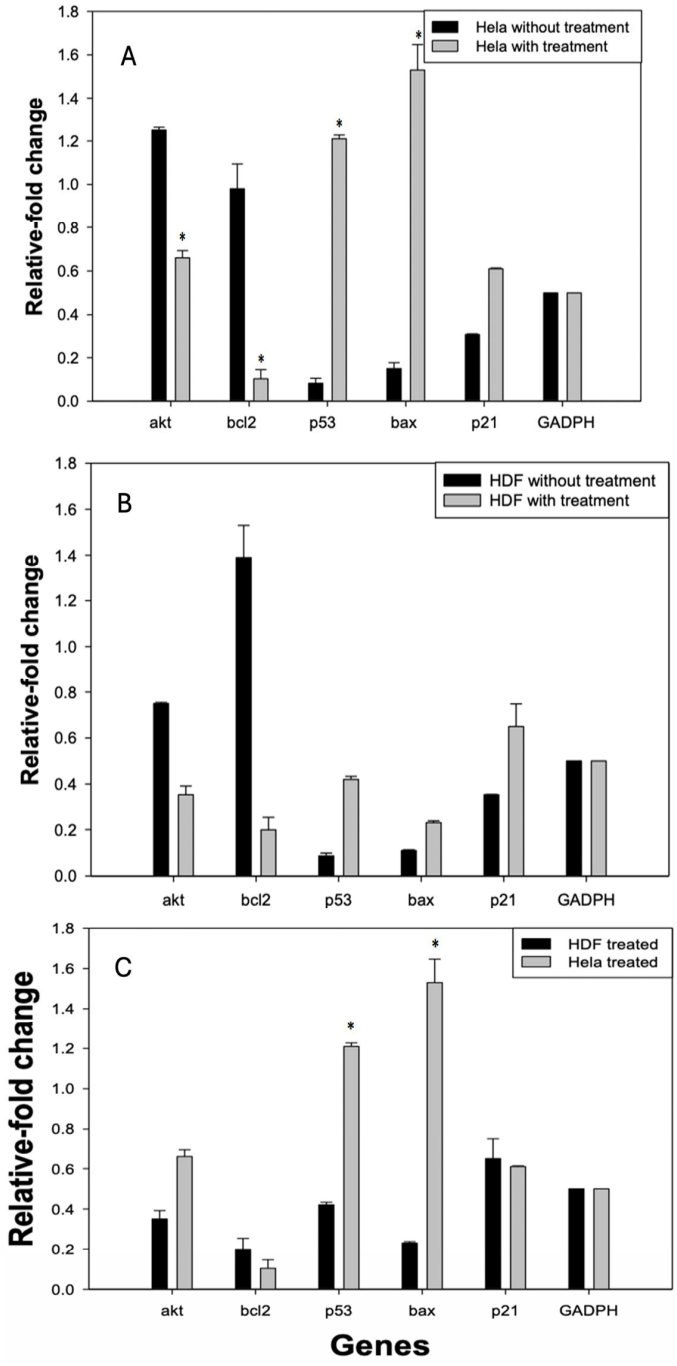

Figure 4. Relative gene expression analysis in RPPE-treated HeLa and HDF cells. HeLa and HDF cells were treated with 175 µg/mL of RPPE for 24 h, and the mRNA expression levels of key regulatory *AKT*, *BCL2*, *P53*, *BAX*, and *P21* genes were quantified by RT-qPCR. Expression levels were normalized to GAPDH and are presented as relative fold changes compared with untreated controls. (**A**)—HeLa cells; (**B**)—HDF cells; (**C**)—Comparative HeLa and HDF cells. Data are presented as mean ± SD of three independent experiments. * Indicates a statistically significant difference ($p < 0.05$).

In HeLa cells, RPPE treatment induced a clear pro-apoptotic shift in gene expression. *P53* expression increased significantly, with a fold change greater than 1.2, suggesting activation of tumor suppressor pathways. *BAX*, a downstream effector of *p53* and a key pro-apoptotic gene, showed the highest upregulation, approaching a 1.6-fold increase, indicating a strong induction of mitochondrial apoptosis.

Conversely, *BCL2* expression was markedly downregulated, consistent with elevated *BAX* levels and disruption of the *BCL2*/*BAX* ratio, which favors apoptotic signaling. *AKT*, a central regulator of cell proliferation and survival, was also suppressed, further contributing to the inhibition of survival pathways. Additionally, *P21*, a cyclin-dependent kinase inhibitor involved in cell-cycle arrest, was modestly upregulated, although to a lesser extent than the apoptotic markers. These findings suggest that while RPPE may induce some degree of cell-cycle arrest, its predominant effect on HeLa cells is the activation of apoptotic pathways. * Indicates a statistically significant difference ($p < 0.05$).

4. Discussion

The present study demonstrated that RPPE selectively reduced the viability of HeLa cervical cancer cells while exerting limited effects on normal HDF cells. A dose- and time-dependent cytotoxic response was observed, with significant reductions in HeLa viability at concentrations $\geq$150 µg/mL. Comparable antiproliferative effects have been reported for plant-derived compounds such as curcumin, quercetin, gallic acid, methyl gallate, and triptolide in HeLa cells [22–24]. However, previous studies evaluating rambutan peel extracts reported limited or no cytotoxic activity against HeLa cells, despite showing activity in other cancer cell lines such as MDA-MB-231 and MG-63 [20,22]. The stronger response observed in the present study may be related to differences in extract composition and extraction methodology. The aqueous rambutan peel extract used herein was previously characterized by a high polyphenol content (307.57 $\pm$ 20.27 mg/g dry matter) and enriched in ellagitannins and related compounds, including corilagin, geraniin, punigluconin, pedunculagin, tetragalloyl glucose, and ellagic acid derivatives [12]. These phytochemicals have been associated with alterations in oxidative stress responses, proliferative activity, and apoptosis-related signaling across different cancer models [17,20,25]. Beyond its biological activity, rambutan peel is an abundant agro-industrial by-product and has been reported to contain higher concentrations of polyphenols than other edible portions of the fruit [20]. Therefore, its valorization as a source of bioactive compounds may simultaneously contribute to waste reduction and the development of sustainable nutraceutical ingredients. Collectively, these findings support further investigation of rambutan peel polyphenols as selective anticancer agents, although additional mechanistic and in vivo studies are required to confirm their therapeutic relevance.

The selective cytotoxic effect of RPPE observed in HeLa cells was further supported by morphological analyses. Compared with untreated controls, RPPE-treated HeLa cells exhibited progressive morphological alterations, including cell shrinkage, loss of intercellular contacts, reduced adherence, cytoplasmic condensation, membrane blebbing, cellular rounding, and accumulation of cellular debris. At the highest concentration tested (200 µg/mL), extensive cell detachment and fragmentation were observed, indicating advanced stages of apoptosis. These observations are consistent with the viability assays and suggest that RPPE-induced growth inhibition is associated with apoptotic cell death. Comparable morphological features have been reported in HOS cells treated with rambutan extract, where cells progressively lost their spindle-shaped morphology and adherence, followed by cellular shrinkage and apoptotic body formation after 72 h of treatment [20]. Likewise, *Sargassum polycystum* extracts induced apoptosis in HeLa cells, accompanied by characteristic morphological alterations and biochemical markers of pro-

grammed cell death [5]. DNA fragmentation, chromatin condensation, and membrane blebbing have also been documented in HeLa cells exposed to gallic acid (GA) and methyl gallate (MG) [22]. Similarly, curcumin has been reported to suppress HeLa cell proliferation by inducing apoptosis and promoting cell-cycle arrest [23,24]. Together, these observations support the involvement of apoptosis-related pathways in the cytotoxic activity of RPPE and reinforce the potential of natural polyphenols as sources of bioactive compounds for cervical cancer research.

At the molecular level, RPPE induced a transcriptional profile consistent with activation of apoptotic pathways and inhibition of cell survival mechanisms in HeLa cells. Treatment upregulated the pro-apoptotic genes *P53* and *BAX* and downregulated the anti-apoptotic and survival-related genes *BCL-2* and *AKT*. In addition, increased *P21* expression suggests concurrent activation of cell-cycle arrest mechanisms, which may contribute to the observed reduction in cell proliferation. These molecular alterations align with the observed reduction in cell viability and the apoptotic morphological features induced by RPPE treatment. Similar pro-apoptotic responses have been reported for other natural polyphenols and rambutan-derived extracts. Previous studies have demonstrated that rambutan-derived polyphenols induce apoptotic responses by shifting the balance between pro-survival and pro-apoptotic signaling pathways, including suppression of Bcl-2 and activation of caspase-mediated cell death mechanisms [25]. Likewise, quercetin, curcumin, gallic acid, and methyl gallate have been shown to induce apoptosis in HeLa cells through coordinated regulation of genes governing cell survival and programmed cell death [22–24]. Although these observations suggest that polyphenols may share common molecular targets, the mechanisms underlying their selective activity in cervical cancer cells remain incompletely understood. To our knowledge, this is among the first studies to characterize the transcriptional response of apoptosis- and survival-related genes in HeLa cells following exposure to a chemically characterized rambutan peel polyphenolic extract, thereby expanding the current understanding of the molecular mechanisms associated with its biological activity.

Beyond its biological activity, rambutan peel has attracted increasing interest as a source of bioactive compounds due to its abundance as an underutilized agro-industrial by-product and its richness in polyphenolic constituents. Among the different anatomical parts of the fruit, the peel contains particularly high concentrations of ellagitannins and related polyphenols, making it a valuable reservoir of bioactive constituents [12,17]. Previous phytochemical studies have identified compounds such as geraniin, corilagin, and ellagic acid derivatives, which have been associated with antioxidant, anti-inflammatory, and anticancer activities in different experimental models [12,16,20]. Furthermore, the use of an aqueous extraction method enhances the sustainability and potential nutraceutical applicability of RPPE. Despite the promising in vitro findings, several limitations should be acknowledged. The biological activity of RPPE was evaluated exclusively in cell culture models and therefore may not fully reflect the complexity of in vivo physiological conditions. Also, the molecular effects were assessed only at the transcriptional level, and additional studies are required to confirm the activation of apoptotic pathways through protein expression and functional analyses. Moreover, although major constituents of RPPE, including geraniin, corilagin, and ellagic acid derivatives, have been associated with beneficial biological effects, hypersensitivity or allergic reactions have been reported in susceptible individuals. Consequently, the safety profile of concentrated rambutan peel preparations requires a comprehensive toxicological evaluation before clinical application can be considered. Future investigations should assess bioavailability, pharmacokinetics, systemic toxicity, immunological responses, and antitumor efficacy in appropriate animal models to determine the translational potential of RPPE.

5. Conclusions

This study demonstrates that rambutan peel polyphenolic extract (RPPE) selectively reduces the viability of HeLa cervical cancer cells while exerting minimal effects on normal fibroblasts. RPPE induced apoptotic morphology and significantly altered the expression of apoptosis-, survival-, and cell-cycle-related genes. Upregulation of *P53*, *BAX*, and *P21*, together with downregulation of *BCL-2* and *AKT*, indicates the activation of pro-apoptotic and growth-inhibitory pathways. To our knowledge, this is among the first reports characterizing transcriptional responses to a chemically defined RPPE in HeLa cells. These findings highlight rambutan peel, an abundant polyphenol-rich by-product, as a sustainable source of anticancer agents. However, the results are limited to in vitro assays, and further studies are needed to identify active constituents, validate mechanisms, assess safety, and confirm antitumor efficacy in animal models before clinical translation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nutraceuticals6030057/s1, Table S1. Primer sequences used for quantitative real-time PCR (qRT-PCR).

Author Contributions: Conceptualization, J.A.M.-C. and P.K.M.-E.; methodology, M.L.M.-F., J.A.M.-C. and P.K.M.-E.; software, M.L.M.-F. and I.G.-V.; validation, M.L.M.-F., J.A.M.-C. and M.A.S.-S.; formal analysis, P.K.M.-E., M.L.M.-F. and J.A.M.-C.; investigation, J.A.M.-C. and P.K.M.-E.; resources, M.L.M.-F. and J.A.-V.; data curation, P.K.M.-E.; writing—original draft preparation, J.A.M.-C., P.K.M.-E. and A.C.C.-N.; writing—review and editing, J.A.M.-C., P.K.M.-E., M.L.M.-F., I.G.-V., M.A.S.-S., J.A.-V., A.C.C.-N., S.S.-N. and R.R.-H.; visualization, S.S.-N.; supervision, R.R.-H.; project administration, M.L.M.-F. and J.A.M.-C.; funding acquisition, M.L.M.-F. and J.A.M.-C. All authors critically reviewed the manuscript, approved the final version for publication, and agree to be accountable for all aspects of the work in accordance with the ICMJE authorship criteria. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Research Ethics Committee of the General Hospital of Saltillo under NOM-012-SSA3-2012 (approval No. 23/2024).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available from the corresponding author upon reasonable request.

Acknowledgments: We would like to give special thanks to Margarita L. Martínez-Fierro from the Molecular Medicine Laboratory in the Autonomous University of Zacatecas, Mexico, where the methodology was entirely developed. The authors are grateful to Juan Ascacio-Valdés for donating the Rambutan peel polyphenolic extract (RPPE) used for the experiments.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. da Costa, S.C.S.; Bonadio, R.C.; Gabrielli, F.C.G.; Aranha, A.S.; Dias Genta, M.L.N.; Miranda, V.C.; de Freitas, D.; Abdo Filho, E.; Ferreira, P.A.O.; Machado, K.K.; et al. Neoadjuvant Chemotherapy with Cisplatin and Gemcitabine Followed by Chemoradiation Versus Chemoradiation for Locally Advanced Cervical Cancer: A Randomized Phase II Trial. *J. Clin. Oncol.* **2019**, *37*, 3124–3131. [CrossRef] [PubMed]
2. Zhu, H.; Zhu, H.; Tian, M.; Wang, D.; He, J.; Xu, T. DNA Methylation and Hydroxymethylation in Cervical Cancer: Diagnosis, Prognosis and Treatment. *Front. Genet.* **2020**, *11*, 347. [CrossRef] [PubMed]
3. Venkatas, J.; Singh, M. Cervical cancer: A meta-analysis, therapy and future of nanomedicine. *Ecancermedicalscience* **2020**, *14*, 1111. [CrossRef] [PubMed]

4. Chatterjee, K.; Mukherjee, S.; Vanmanen, J.; Banerjee, P.; Fata, J.E. Dietary Polyphenols, Resveratrol and Pterostilbene Exhibit Antitumor Activity on an HPV E6-Positive Cervical Cancer Model: An In Vitro and In Vivo Analysis. *Front. Oncol.* **2019**, *9*, 352. [CrossRef] [PubMed]
5. Firdaus, M.; Setijawati, D.; Islam, I.; Nursyam, H.; Kartikaningsih, H.; Yufidasari, H.S.; Prihanto, A.A.; Nurdiani, R.; Jaziri, A.A. The reducibility of heLa cell viability by Sargassum polycystum extracts. *IOP Conf. Ser. Earth Environ. Sci.* **2018**, *137*, 012064. [CrossRef]
6. Wang, G.; Luo, Y.; Yang, J.; Hou, C.; Li, J. Inhibitory effects of polyphenols-enriched extracts from Debregeasia orientalis leaf against human cervical cancer in vitro & in vivo. *Food Agric. Immunol.* **2020**, *31*, 176–192. [CrossRef]
7. Sanlier, N.T.; Saçinti, K.G.; Türkoğlu, İ.; Sanlier, N. Some Polyphenolic Compounds as Potential Therapeutic Agents in Cervical Cancer: The Most Recent Advances and Future Prospects. *Nutr. Rev.* **2025**, *83*, 880–896. [CrossRef] [PubMed]
8. Xiao, Y.; Yin, J.; Liu, P.; Zhang, X.; Lin, Y.; Guo, J. Triptolide-induced cuproptosis is a novel antitumor strategy for the treatment of cervical cancer. *Cell. Mol. Biol. Lett.* **2024**, *29*, 113. [CrossRef] [PubMed]
9. Zhang, Z.; Chen, B.; Liu, Y.; Zhang, K.; Wei, Z.; Dai, Y.; Liu, C. Advances in Nanomedicine for Targeting Cancer Stem Cells and Overcoming Therapeutic Resistance. *ACS Nano* **2025**, *19*, 30720–30757. [CrossRef] [PubMed]
10. Tao, T.; Zhang, P.; Zeng, Z.; Wang, M. Advances in autophagy modulation of natural products in cervical cancer. *J. Ethnopharmacol.* **2023**, *314*, 116575. [CrossRef] [PubMed]
11. Dretcanu, G.; Iuhas, C.I.; Diaconeasa, Z. The Involvement of Natural Polyphenols in the Chemoprevention of Cervical Cancer. *Int. J. Mol. Sci.* **2021**, *22*, 8812. [CrossRef] [PubMed]
12. Hernández-Hernández, C.; Estrada-Gil, L.E.; Lozano-Sepúlveda, S.A.; Rivas-Estilla, A.M.; Govea-Salas, M.; Morlett-Chávez, J.; Aguilar, C.N.; Ascacio-Valdés, J.A. Antiviral Activity of Rambutan Peel Polyphenols Obtained Using Green Extraction Technology and Solvents. *Sustain. Chem.* **2025**, *6*, 14. [CrossRef]
13. Hernández-Hernández, C.; Aguilar, C.N.; Flores-Gallegos, A.C.; Sepúlveda, L.; Rodríguez-Herrera, R.; Morlett-Chávez, J.; Govea-Salas, M.; Ascacio-Valdés, J. Preliminary Testing of Ultrasound/Microwave-Assisted Extraction (U/M-AE) for the Isolation of Geraniin from *Nephelium lappaceum* L. (Mexican Variety) Peel. *Processes* **2020**, *8*, 572. [CrossRef]
14. Perumal, A.; AlSalhi, M.S.; Kanakarajan, S.; Devanesan, S.; Selvaraj, R.; Tamizhazhagan, V. Phytochemical evaluation and anticancer activity of rambutan (*Nephelium lappaceum*) fruit endocarp extracts against human hepatocellular carcinoma (HepG-2) cells. *Saudi J. Biol. Sci.* **2021**, *28*, 1816–1825. [CrossRef] [PubMed]
15. Afzaal, M.; Saeed, F.; Bibi, M.; Ejaz, A.; Shah, Y.A.; Faisal, Z.; Ateeq, H.; Akram, N.; Asghar, A.; Shah, M.A. Nutritional, pharmaceutical, and functional aspects of rambutan in industrial perspective: An updated review. *Food Sci. Nutr.* **2023**, *11*, 3675–3685. [CrossRef] [PubMed]
16. Liu, Q.; Ma, Q.; Li, J.; Sun, L.; Zhuang, Y. Hypoglycemic effect of rambutan (*Nephelium lappaceum* L.) peel polyphenols on type 2 diabetes mice by modulating gut microbiota and metabolites. *Mol. Nutr. Food Res.* **2024**, *68*, 2400555. [CrossRef] [PubMed]
17. Jantapaso, H.; Mittraparp-Arthorn, P. Phytochemical Composition and Bioactivities of Aqueous Extract of Rambutan (*Nephelium lappaceum* L. cv. Rong Rian) Peel. *Antioxidants* **2022**, *11*, 956. [CrossRef] [PubMed]
18. Torgbo, S.; Rugthaworn, P.; Sukatta, U.; Sukyai, P. Biological Characterization and Quantification of Rambutan (*Nephelium lappaceum* L.) Peel Extract as a Potential Source of Valuable Minerals and Ellagitannins for Industrial Applications. *ACS Omega* **2022**, *7*, 34647–34656. [CrossRef] [PubMed]
19. Isacfranklin, M.; Yuvakkumar, R.; Ravi, G.; Kumar, P.; Saravanakumar, B.; Velauthapillai, D.; Alahmadi, T.A.; Alharbi, S.A. Biomedical application of single anatase phase TiO_2 nanoparticles with addition of Rambutan (*Nephelium lappaceum* L.) fruit peel extract. *Appl. Nanosci.* **2020**, *11*, 699–708. [CrossRef]
20. Hidayati, W.S.W.N.; Ridzuan, P.M.; Seeni, A. *Nephelium lappaceum* rind as a new chemopreventive agent: Arresting the cell cycle at G2/M and promoting apoptotic cell death in vitro on osteosarcoma cell lines. *Int. J. Med. Toxicol. Leg. Med.* **2019**, *22*, 6–12. [CrossRef]
21. Tingting, Z.; Xiuli, Z.; Kun, W.; Liping, S.; Yongliang, Z. A review: Extraction, phytochemicals, and biological activities of rambutan (*Nephelium lappaceum* L) peel extract. *Heliyon* **2022**, *8*, e11314. [CrossRef] [PubMed]
22. Abdullah, H.; Ismail, I.; Suppian, R.; Zakaria, N.M. Natural Gallic Acid and Methyl Gallate Induces Apoptosis in Hela Cells through Regulation of Intrinsic and Extrinsic Protein Expression. *Int. J. Mol. Sci.* **2023**, *24*, 8495. [CrossRef] [PubMed]
23. Fatima, S.; Borappa, M.; Kanakarajan, S.; Selvaraj, R.; Siddiqi, N.J.; Wasi, S.; Siyal, A.; Patel, N.; Sharma, P. Apoptosis, DNA damage, and cell cycle arrest: The anticancer mechanisms of quercetin in human cervix epithelioid carcinoma cells. *Int. J. Health Sci.* **2025**, *19*, 43–51. [CrossRef] [PubMed]

24. Purba, I.S.; Irwanto, Y.; Rahardjo, B.; Handayani, P. The Effect of Curcumin Administration on p53 and Caspase-3 Expression in Cervical Cancer HeLa Cell Culture. *Asian J. Health Res.* **2024**, *3*, 228–232. [CrossRef]
25. Utami, W.P.; Tallei, T.E.; Turalaki, G.L.A.; Tendean, L.E.N.; Kaseke, M.M.; Purwanto, D.S. Targeting Prostate Cancer with Rambutan Peel-Derived Compounds via Network Pharmacology. *Malacca Pharm.* **2025**, *3*, 42–49. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.