

Integrated Cellular and Molecular Interaction Analysis of Turmeric and Black Rice Bran Extract in HeLa Cells as a Potential Anticancer

Rantika Silfarohana^{1,2}, Rizal Maarif Rukmana³, Muhammad Saifur Rohman⁴, Sari Haryanti³, Weka Sidha Bhagawan^{5*}

¹Research Center for Vaccine and Drug, Research Organization for Health, National Research and Innovation Agency (BRIN), Bogor, West Java, 16911, Indonesia

²Master Program of Biotechnology, Graduate School, Universitas Gadjah Mada, Sleman, Yogyakarta, 55281, Indonesia

³Research Center for Pharmaceutical Ingredients and Traditional Medicine, Research Organization for Health, National Research and Innovation Agency (BRIN), Bogor, West Java, 16911, Indonesia

⁴Department of Agricultural Microbiology, Faculty of Agriculture, Universitas Gadjah Mada, Sleman, Yogyakarta, 55281, Indonesia

⁵Department of Pharmacy, Faculty of Health and Sciences, Universitas PGRI Madiun, Madiun, East Java, 63118, Indonesia

*Corresponding author: weka.sidha@unipma.ac.id

Abstract

Cervical cancer continues to be a leading cause of female mortality due to multidrug resistance therapy associated with EGFR overexpression and evasion of apoptosis. Turmeric and black rice bran are recognized for their potential anticancer properties; however, the combined effects of these extracts and their predicted molecular interactions remain to be elucidated. This study investigated the potential enhanced effect of combined turmeric and black rice bran extracts using an integrated phenotypic and molecular docking approach. The antiproliferative effect of the combination of the ethanolic extract of turmeric and black rice bran was assessed in HeLa cells using the MTT assay, whereas cell cycle arrest and apoptosis were analyzed using flow cytometry. Several compounds in turmeric and black rice bran extract were docked against the growth signaling protein (EGFR) and death receptors (BCL-2, DR-4). The optimal formulation (Combination 1) demonstrated a progressive reduction in HeLa cell viability from 61.02% at 24 hours to 47.88% at 48 hours, inducing a distinct G2/M phase arrest. This growth inhibition was accompanied by a cell clearance through early apoptosis (38.57%) and secondary necrosis (28.03%). Molecular docking supported these findings by suggesting that bisdemethoxycurcumin may interact with EGFR, with a binding affinity of -7.6 kcal/mol. Compounds derived from black rice bran were also predicted to complementarily target BCL-2 and DR-4, suggesting a potential multi-target mechanism. In summary, the combination extract demonstrates both growth signaling and apoptotic thresholds, offering a promising baseline for further validation in *in vivo* models.

Keywords

Cervical Cancer, Turmeric, Black Rice Bran, Combination Effect, HeLa Cell

Received: 2 March 2026, Accepted: 27 June 2026

<https://doi.org/10.26554/sti.2026.11.4.1274-1286>

1. INTRODUCTION

Cervical cancer remains a leading cause of female mortality globally, with its prognosis frequently compromised by drug resistance linked to epidermal growth factor receptor (EGFR) overexpression and apoptosis evasion (Du et al., 2025; Oh et al., 2023). Conventional chemoradiation remains the standard treatment, but systemic toxicity and cellular resistance often hinder its clinical efficacy (Lin et al., 2025). Consequently, investigating plant-derived dietary phytochemicals has emerged as a viable strategy to enhance therapeutic sensitivity and overcome resistance mechanisms in malignant cells (Marema et al., 2026; Sevindik et al., 2025). Among these, *Curcuma longa* L. and black rice bran (*Oryza sativa* L.) have attracted attention due to their distinct anticancer profiles; turmeric-derived curcum-

inoids are well-documented to suppress tumor proliferation and disrupt standard cell cycle progression (Hu et al., 2017; Zhang et al., 2023), while black rice bran anthocyanins, such as cyanidin-3-glucoside, are potent activators of programmed cell death pathways (Huang and Lai, 2016; Rukmana et al., 2016).

Although the individual therapeutic benefits of these compounds are established, their evaluation in combination remains strictly limited. Combining these extracts provides a strategic rationale for a multi-target therapeutic approach. Specifically, curcumin can be leveraged to block upstream proliferation signals by targeting EGFR (Chen et al., 2015). At the same time, the phenolic and anthocyanin matrix of black rice bran can simultaneously lower apoptotic thresholds by engaging

apoptotic regulators (Pratiwi et al., 2015). While a preliminary study by Silfarohana et al. (2025) noted the phenotypic cytotoxicity of this combination, the underlying molecular coordination and its dual engagement with EGFR, BCL-2, and DR-4 have not been integratively assessed.

Mechanistically, cervical cancer development is mediated by the involvement of various signaling pathways that interact to increase proliferation and inhibit apoptosis. The EGFR gene drives several PI3K/Akt and MAPK signaling pathways that can stimulate cell proliferation, the cell cycle, and therapeutic resistance (Roberts et al., 2025). Continuous activation of these signaling pathways increases the level of antiapoptotic proteins, such as BCL-2, thereby inhibiting apoptosis (Ilyas and Lee, 2025; Vogler et al., 2025). Conversely, activation of death receptors, such as DR-4, stimulates the extrinsic apoptosis pathway by activating the caspase cascade (Rahman et al., 2022). Disruption of the balance between these pathways allows cancer cells to evade programmed cell death while maintaining uncontrolled growth. Simultaneous targeting of EGFR, BCL-2, and DR-4 has the potential to enhance anticancer efficacy by inhibiting proliferative signals and promoting apoptosis (Roberts et al., 2025).

Recent advancements in phytopharmacology suggest that mixtures of bioactive plant extracts may offer greater efficacy than single-compound therapies, owing to their complementary, multi-target mechanisms of action (Pebriani et al., 2026). In contrast to traditional monotherapies that often target a single biological entity, phytochemical combinations can concurrently modulate multiple signaling pathways implicated in cancer progression (Akinwumi and Ambali, 2026; Sitarek et al., 2020). This strategy is especially pertinent for cervical cancer, as resistance mechanisms emerge from redundant survival pathways (Nurani et al., 2025; Rukmana et al., 2016). The combination of turmeric and black rice bran is posited to provide potential enhanced effects by concurrently inhibiting proliferative signaling and augmenting apoptotic responsiveness, thereby potentially diminishing the likelihood of adaptive resistance. This study evaluates the combination of turmeric and black rice bran on cervical cancer cells *in vitro* as inhibitors of cell proliferation, cell cycle arrest, and apoptosis and links these effects to molecular interactions with EGFR, BCL-2, and DR-4 through molecular docking.

2. EXPERIMENTAL SECTION

2.1 Materials

Turmeric (*Curcuma longa* L.) was sourced from farmers of the “Sentra Tanaman Obat” in Karanganyar, Central Java, Indonesia, and “Sembada Hitam” black rice (*Oryza sativa* L.) was cultivated in Sleman, Yogyakarta, Indonesia. The Standardization Laboratory for Traditional Medicine Raw Materials, National Research and Innovation Agency (BRIN), located in Tawangmangu, provided HeLa ATCC CCL-2 cell lines. Annexin V-FITC, dimethyl sulfoxide (DMSO), fetal bovine serum (FBS), flow cytometry reagents, HEPES, fungizone, MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium

bromide] reagent, penicillin-streptomycin (P/S), phosphate-buffered saline (PBS), propidium iodide (PI), RNase, RPMI 1640 powder, sodium dodecyl sulfate (SDS), and Triton X-100 were purchased from Sigma Aldrich, St. Louis, Missouri. Filter paper and 96% ethanol were obtained from Darmstadt-based Merck Life Science.

2.2 Methods

2.2.1 Preparation and Extraction of Turmeric and Black Rice Bran Extract

The turmeric rhizome and black rice bran samples were cleaned, sliced, and dried in an oven at 35–40 °C until a uniform weight was achieved. Each sample was ground and passed through a 40-mesh sieve. Sixty grams of turmeric powder and 500 grams of black rice bran were macerated in 96% ethanol (1:10 w/v) for two 24-hour periods, with periodic stirring. The resulting filtrate was collected and filtered through Whatman No. 1 paper (Silfarohana et al., 2025).

2.2.2 Antiproliferation Assay

HeLa cells were cultured in RPMI Medium 1640 supplemented with 10% (v/v) FBS, 1% (v/v) P/S, and 0.5% (v/v) fungizone. HeLa cells were cultured in a 25 cm² T-flask and incubated in a 5% CO₂ incubator at 37 °C (Wang et al., 2017). Three combination extracts from the previous study Silfarohana et al. (2025) were used in the antiproliferation assay. The combination includes Combination 1 ($\frac{1}{2}$ IC₅₀ of turmeric + $\frac{1}{2}$ IC₅₀ Black Rice Bran), Combination 2 ($\frac{1}{2}$ IC₅₀ of turmeric + $\frac{1}{4}$ IC₅₀ Black Rice Bran), and Combination 3 ($\frac{1}{2}$ IC₅₀ of turmeric + $\frac{1}{16}$ IC₅₀ Black Rice Bran) in three repetitions. In the study, turmeric extract had an IC₅₀ of 54 µg/mL, while black rice bran had an IC₅₀ of 446 µg/mL. The antiproliferation test of the three combination extracts was conducted using the MTT assay with incubation times of 0, 24, and 48 hours (Chen et al., 2015). The cell viability is calculated using Equation 1 (Zamrus et al., 2018).

$$\text{Cell viability (\%)} = \frac{\text{Treated cell absorbance} - \text{Media control absorbance}}{\text{Cell control absorbance} - \text{Media control absorbance}} \times 100\% \quad (1)$$

2.2.3 Cell Cycle Arrest Assay

A cell cycle arrest assay was conducted using the following treatments: cell control, DMSO control, Combination 1, Combination 2, and Combination 3. Every treatment was assessed in three replications. The HeLa cells were concentrated at 5×10^5 cells, distributed 1000 µL into a six-well plate, and then incubated for 24 hours in an incubator (37 °C, 5% CO₂, 95% humidity). Subsequently, 500 µL of the combination extracts was added to the wells, while the DMSO control was prepared at 0.4% and added to the culture medium. After the treatments, the cells were re-incubated for 24 hours (Martinotti and Ranzato, 2020; Rukmana et al., 2017).

By adding 150 µL of 0.25% trypsin-EDTA and 1000 µL of culture medium, the cells were harvested, resuspended, and collected into conical tubes. Afterward, 500 µL of 70% cold

ethanol was added to the cell, which was then incubated for 30 minutes at 37 °C to enable the fixation. The samples were centrifuged at 2000 rpm for 3 minutes, and the supernatant was removed. Aluminum foil was used to cover the samples, flow cytometry reagents were added (25 μ L PI + 1 μ L RNase + 0.5 μ L Triton X-100 + 500 μ L PBS for each sample), incubated for 30 minutes, and then analyzed using FACS Calibur flow cytometry (Miranda et al., 2025), where a minimum of 10,000 events per sample were collected. The gating strategy employed initial forward scatter (FSC) versus side scatter (SSC) gating to exclude cell debris. Subsequently, doublet discrimination was performed to ensure that only single-cell populations were analyzed for cell cycle distribution and apoptotic phases (Nair and Manohar, 2021).

2.2.4 Apoptosis Assay

The apoptosis assay was conducted using the same treatments as the cell cycle arrest analysis. A six-well plate was added with 5×10^5 HeLa cells with a volume of 1000 μ L/well. The samples were subjected to the same treatments in the cell cycle arrest assay. The HeLa cells were incubated at 37 °C, 5% CO₂, and 95% humidity. After 24 hours of incubation, the cells were washed with 1000 μ L of 1X PBS, and the treatments were then administered. The cells were incubated for 48 hours, then harvested with 0.25% trypsin-EDTA, and resuspended in 1000 μ L of culture media. Subsequently, 100 μ L of binding buffer and 10 μ L of Annexin V-FITC were added to the samples, which were incubated at room temperature for 15 minutes. The samples were washed with binding buffer, added with 5 μ L of PI solution, and then analyzed by FACS Calibur flow cytometry (Kania et al., 2025).

2.2.5 Analysis of Bioavailability and Toxicity

Bioavailability analysis was performed online with the web-based program SCFBio (<http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp>). The analysis aimed to ascertain the permeability and solubility of a substance within the body by evaluating Lipinski's rules. These criteria encompass molecular mass, hydrogen bond donors, hydrogen bond acceptors, logP, and molar refractivity (Lipinski, 2004). In accordance with the Lipinski guidelines, the prospective chemicals derived from turmeric and black rice bran extracts were subsequently evaluated for potential toxicity.

Toxicity analysis was conducted through the website <https://biosig.lab.uq.edu.au/pkcsmprediction>. This analysis involves inputting the SMILES code of each compound and generating predictions based on various parameters accessible within the program. The analyzed metrics include carcinogenicity (AMES toxicity), suppression of the Human Ether A-Go-Go Related Gene (hERG), acute oral toxicity (LD₅₀), and the minnow test (Rahman et al., 2022).

2.2.6 Molecular Docking

The phytochemicals from turmeric and black rice bran extract were analyzed by molecular docking simulations, focus-

ing on EGFR, BCL-2, and DR4 as their respective receptors. The three-dimensional structures of the substances were obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>), and those not available in the database were generated using Avogadro 1.2.0. Simultaneously, the receptors were obtained from the Protein Data Bank (<https://www.rcsb.org/>), namely EGFR (PDB ID: 1M17) at 2.60 Å resolution, BCL-2 (PDB ID: 2XA0) at 2.70 Å resolution, and DR-4 (PDB ID: 1DG6) at 1.30 Å resolution.

The molecular docking procedure was conducted utilizing Chimera UCSF in conjunction with AutoDock Vina. This analysis involves ligand and receptor production, which involves solvent removal, hydrogen addition, charge assignment, and docking. LigPlot enabled the two-dimensional representation of residue interactions, whereas three-dimensional visualizations were produced utilizing PyMol 3.1.3 (Rukmana et al., 2025).

2.2.7 Data Analysis

The anti-proliferation assay provided data on cell viability following treatment. The flow cytometry results for the cell cycle test indicated the percentage distribution of the cell cycle, whereas the apoptosis test revealed the proportion of cell types in each quadrant. All test data were subsequently analyzed using one-way analysis of variance (ANOVA). If the data indicated a significant difference at the alpha 0.05 level, the Honestly Significant Difference (HSD) Tukey test and Duncan's multiple range test (DMRT) were employed for antiproliferation assessments, cell cycle arrest, and apoptosis evaluations.

3. RESULTS AND DISCUSSION

3.1 Antiproliferation Activity

Three combination extracts were administered to HeLa cell lines to analyze the proliferation inhibition using the MTT assay. Figure 1 illustrates the viability of HeLa cells after being administered three combination extracts with incubation times of 0, 24, and 48 hours. Overall, it is shown that HeLa cell viability decreased with increasing incubation time after treatment across all extract combinations. Combination 1 showed the lowest cell viability, whereas Combination 3 had the highest living cell count at the final incubation time.

Combination 1 exhibited a reduction in cell viability from 61.02% at 24 h to 47.88% at 48 h of incubation. Combination 2 decreased living cells from 63.95% to 59.76%, and Combination 3 from 69.22% to 61.21% during the 24 h to 48 h incubation, respectively. Combination 1 showed the highest antiproliferative effect among the combinations, with a 13% decline. The anticancer activity observed in Combination 1 demonstrated a dose-reduction effect. Compared with the study by Kurniawan et al. (2016), turmeric extract inhibited HeLa cells' growth by only 50% with a concentration of 184.5 μ g/mL. Meanwhile, the black rice bran extract required a concentration of 408.13 μ g/mL (Pratiwi et al., 2015) to reduce cell viability by 50%. This study revealed the antiproliferative effect at 47.88% only using a half concentration ($\frac{1}{2}$ IC₅₀ turmeric

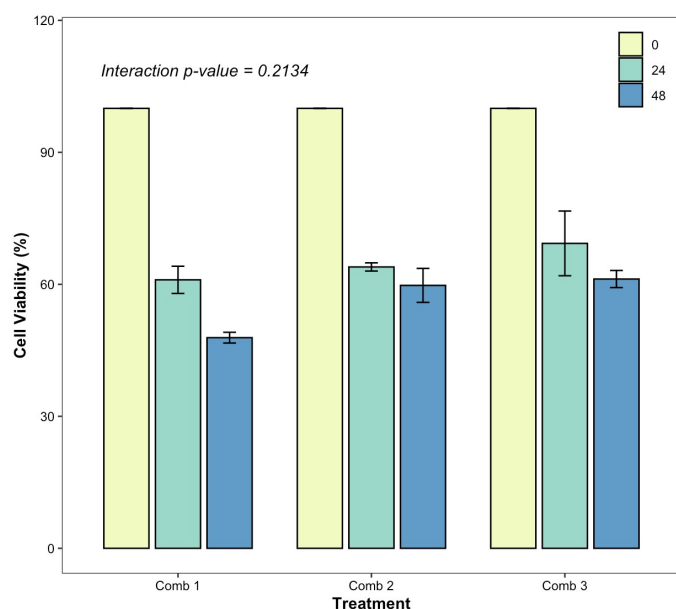


Figure 1. The Antiproliferative Effect of the Combination Extracts on HeLa Cells. Values are Presented as Mean $\pm$ SD ($n = 3$ Replications). Statistical Analysis was Conducted Utilizing One-Way ANOVA, Followed by HSD Tukey ($\alpha = 0.05$). Combination 1 ($\frac{1}{2}$ IC₅₀ of Turmeric + $\frac{1}{2}$ IC₅₀ Black Rice Bran), Combination 2 ($\frac{1}{2}$ IC₅₀ of Turmeric + $\frac{1}{4}$ IC₅₀ Black Rice Bran), and Combination 3 ($\frac{1}{2}$ IC₅₀ of Turmeric + $\frac{1}{16}$ IC₅₀ Black Rice Bran)

extract at 27 $\mu\text{g/mL}$ and $\frac{1}{2}$ IC₅₀ black rice bran extract at 223 $\mu\text{g/mL}$). This finding suggests that the combination of turmeric and black rice bran extract exhibits greater cytotoxic activity than the individual extracts, potentially reducing the therapeutic threshold required to induce cell death in HeLa cells.

Conversely, a reduction in the concentration of black rice bran extract in Combination 2 and Combination 3 caused a decrease in the antiproliferative effect. In Combination 2 and Combination 3, where the concentration of black rice bran was reduced to one-quarter (111.5 $\mu\text{g/mL}$) and one-sixteenth (55.75 $\mu\text{g/mL}$) of the IC₅₀ concentration, respectively, the viability of HeLa cells only decreased to 59.76% and 61.21% after 48 hours. This reduction in black rice bran extract concentration was associated with a corresponding decrease in antiproliferative effect. This dose-dependent trend suggests that black rice bran extract may contribute to the combination's increased activity.

In addition, Combination 3 showed a minimal decrease in viability over the incubation period compared with the other combinations. It suggests that at lower concentrations, the compounds derived from black rice bran extract are insufficient to sustain suppression against HeLa cell proliferation. This trend further implies that black rice bran phytochemicals may act as essential sensitizing agents, required at a specific threshold

to prevent cancer cells from overcoming the growth-inhibitory effects of the turmeric extract. This sensitizing potential aligns with earlier findings by Pratiwi et al. (2015), which established the inherent cytotoxicity of black rice bran extract against HeLa cells, thereby validating its crucial role in driving the observed combinatorial efficacy at optimal doses. Further analysis is required to determine whether the combination extract induces cell-cycle arrest or directly triggers programmed cell death pathways.

3.2 Cycle Cell Arrest of HeLa Cell Lines

Cell cycle tests were conducted on HeLa cells treated with several types of extract treatments. The treatments included cell control, DMSO control, Combination 1, Combination 2, and Combination 3. This test was conducted using flow cytometry to ascertain alterations in the distribution of HeLa cell cycle phases. Table 1 presents the distribution of cell percentages across the various phases of the cell cycle, while Figure 2 provides graphical representations of the data.

The flow cytometry analysis revealed that both the cell control and the DMSO control exhibited a substantial percentage of cells in the G1 phase, at 57.93% and 53.96%, respectively, signifying that over half of the total cells are normal and undergoing uninterrupted cell division. The G1 phase is a stage in which the cell proliferates and synthesizes proteins before DNA replication (Bertoli et al., 2013). In contrast, the distribution of HeLa cells decreased after administration of Combination 1 (46.97%), Combination 3 (40.6%), and Combination 3 (37.63%) in that phase. The significant reduction observed in the G1 phase indicated that the combination extracts influenced the cell's transition to the subsequent phase.

Interestingly, instead of being accumulated in the S phase for DNA replication, the cells in this stage were regulated by the ratio of the combination extract. Combination 1 managed to limit the S-phase population to just 10.70%, whereas combinations 2 and 3 showed higher accumulations at 15.37% and 13.97%, respectively. This trend suggests that the turmeric extract may suppress the DNA synthesis in upstream growth regulation via EGFR signaling (Liang et al., 2021; Saeed et al., 2022), while the presence of black rice bran extract strengthens the blockade by inducing apoptosis (Pratiwi et al., 2015). Conversely, the concentration of black rice bran extract was reduced in Combination 2 and 3, leading to a relaxed translational check. Consequently, a higher proportion of cells transitioned from the G1 phase to the S phase, facilitating DNA synthesis. However, this unexpected containment in the S phase implies that the major bulk of the arrested population, particularly in Combination 1, must have accumulated in the subsequent phase of the cycle.

The G2/M phase showed an accumulated bottleneck of the combination extracts compared to the cell control and the DMSO control. Combination 3 demonstrated the highest cell population (42.17%), followed by combination 2 (36.43%) and combination 1 (34.90%). In contrast, the cell control and the DMSO cell exhibited percentages of 27.67% and 27.47%, re-

Table 1. The Distribution of HeLa Cells Following the Application of the Combination Extracts

Treatment	Distribution of HeLa Cells (%)				
	SubG ₁	G ₁	S	G ₂ /M	Polyploidy
Cell Control	6.5 ± 0.26 ^{cd}	57.93 ± 0.78 ^a	7.77 ± 0.25 ^e	27.67 ± 0.32 ^c	0.17 ± 0.006 ^a
DMSO Control	9.8 ± 0.10 ^b	53.96 ± 1.45 ^b	8.63 ± 0.21 ^e	27.47 ± 1.44 ^c	0.17 ± 0.12 ^a
Combination 1	7.17 ± 0.86 ^{cd}	46.97 ± 1.76 ^c	10.7 ± 0.70 ^d	34.9 ± 3.24 ^b	0.2 ^a
Combination 2	7.37 ± 0.67 ^c	40.6 ± 1.66 ^d	15.37 ± 0.85 ^b	36.43 ± 3.09 ^b	0.2 ^a
Combination 3	6.1 ± 0.35 ^d	37.63 ± 0.23 ^e	13.97 ± 0.59 ^c	42.17 ± 0.42 ^a	0.17 ± 0.06 ^a

*Statistical analysis was conducted for each column utilizing one-way ANOVA, followed by HSD Tukey and DMRT tests ($\alpha = 0.05$). The identical letters in the same column show no significant difference.

Table 2. The Bioavailability Analysis of Turmeric Compounds According to the Lipinski Rule's

Compound	MW (Dalton)	LogP (< 5)	Hydrogen Donor (< 5)	Hydrogen Acceptor (< 10)	Molar Refractivity (40–130)
(+)-ar-turmerone	216	4.024	0	1	68.649
Bisdemethoxycurcumin	308	3.353	2	4	88.912
Curcumin	368	3.370	2	6	102.016
Indole	117	2.168	1	0	38.299

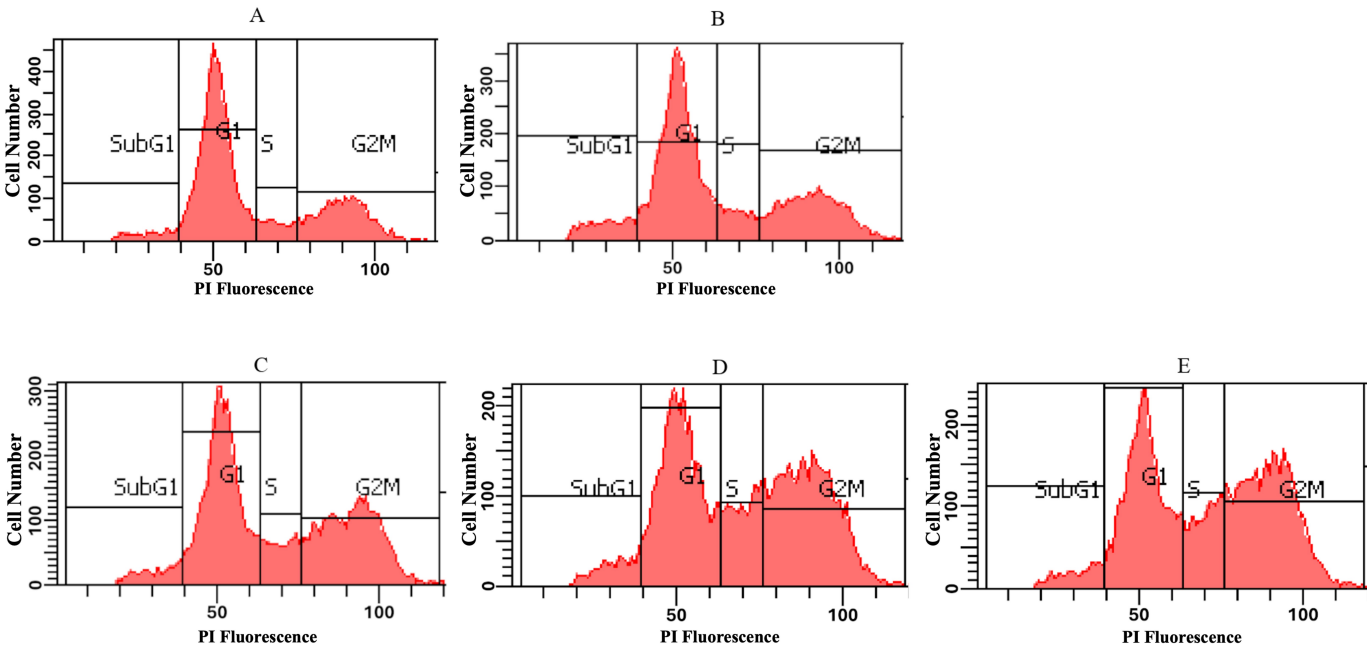


Figure 2. The Results of the Cell Cycle Arrest Analysis of HeLa Cells Using Flow Cytometry. Caption A: Cell Control, B: DMSO Control, C: Combination 1 ($\frac{1}{2}$ IC₅₀ of Turmeric + $\frac{1}{2}$ IC₅₀ Black Rice Bran), D: Combination 2 ($\frac{1}{2}$ IC₅₀ of Turmeric + $\frac{1}{4}$ IC₅₀ Black Rice Bran), and E: Combination 3 ($\frac{1}{2}$ IC₅₀ of Turmeric + $\frac{1}{16}$ IC₅₀ Black Rice Bran)

spectively. This pattern suggests that the combination extract ratio had a distinct impact on the cycle cell arrest. Combination 3, characterized by a reduced dose of black rice bran extract, may elicit a combined effect that is merely adequate to achieve a sustained growth arrest. This pressure may impede the cells at the G2/M checkpoint, hindering their prompt elimination. In contrast, the optimal concentration of black rice bran extract

in combination 1 may induce cell cycle arrest beyond the survival threshold, leading to cell death. As a result, these dying cells undergo fragmentation and exit the active cell cycle gates, resulting in a lower count of intact cells in the G2/M phase for combination 1. This strategic blockade at the downstream G2/M molecular checkpoint suggests a potential correlation with established

treatment mechanisms, which are theoretically characterized by cellular stress either inactivating the CDK1-Cyclin B complex or activating p53-p21 cascades to inhibit mitotic entry (Chen, 2016; Zhang et al., 2020), and is often caused by the damage of intracellular DNA (Lezaja and Altmeyer, 2018). While direct experimental quantification of these specific signaling proteins was not conducted in the present study, these literature-driven mechanisms provide a plausible biochemical framework to explain the phenotypic growth suspension observed in HeLa cells.

In this study, it probably occurred due to the presence of the combination extract. The improved arrest and subsequent cell clearance noted here may be influenced by the phytochemical synergy between turmeric curcuminoids and black rice bran anthocyanins (Hyun and Chung, 2004). The shift from an extended cell cycle arrest to swift clearance in combination 1 necessitates a thorough investigation of the precise mechanism of cell death. To determine if this accumulation pattern causes definitive programmed cell death or a transient growth arrest, a thorough apoptosis investigation was conducted.

3.3 Apoptosis of HeLa Cell Lines

Three combination extracts were tested in HeLa cells to assess their ability to induce apoptosis within 24 hours of incubation. The results of the apoptosis-induction study for the treatments are depicted in Figures 3 and 4. The data demonstrated that the combination extracts reduced the number of viable HeLa cells.

The assessment of apoptotic activity clearly demonstrated that the process occurred in HeLa cells following cell cycle arrest, thereby providing a comprehensive conclusion to the kinetics of growth inhibition. Combination 1 resulted in a significant reduction of the viable cell fraction to 31.53%, which correlates with early apoptosis (38.57%), late apoptosis (1.83%), and necrosis (28.03%). Combination 2 demonstrated viable cells at a rate of 73.1%, early apoptosis at 11.53%, late apoptosis at 3.23%, and necrosis at 12.17%. Meanwhile, in Combination 3, with the lowest doses of black rice bran extract, resulted in viable cells, early apoptosis, late apoptosis, and necrosis at 88.77%, 4.37%, 2.07%, and 4.8%, respectively.

The apoptotic profile of combination 1 exhibited a distinctive pattern, characterized by a substantial proportion of early apoptotic cells (38.57%) and a notable necrotic population (28.03%), while late apoptotic cells were present in minimal quantities (1.83%). This distinctive pattern suggests accelerated cell death kinetics. The transition from early programmed cell death to terminal disintegration may occur rapidly as a result of the combined actions of turmeric and black rice bran extract at appropriate ratios. After entering the apoptotic cascade, HeLa cells undergo rapid membrane degradation, swiftly progressing through late apoptosis and ultimately presenting as secondary necrosis at 48 hours of incubation (Sachet et al., 2017).

The elevated necrotic readout in combination 1 is not indicative of non-specific, erratic toxicity; instead, it suggests a progressive transition toward secondary necrosis following an

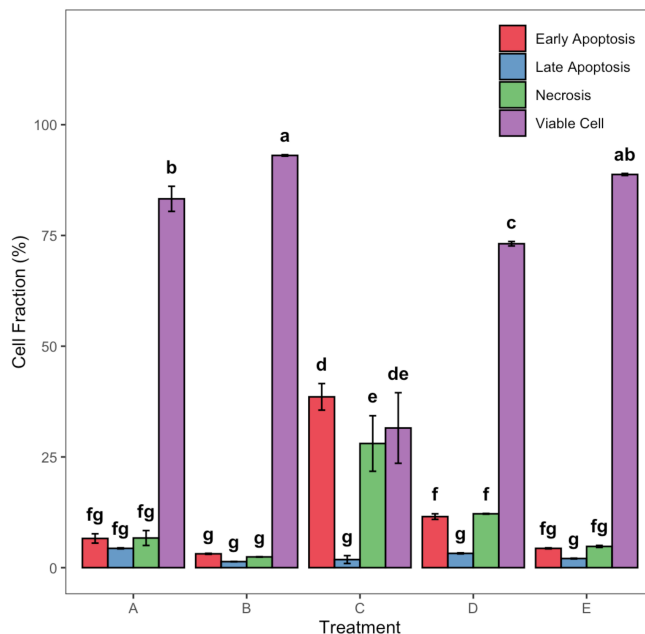


Figure 3. Percentage Distribution of Fraction Cells After Treatment. Data are Presented as Mean $\pm$ SD in 3 Replications. Statistical Analysis Was Conducted Using One-Way ANOVA, Followed by Tukey's HSD and DMRT Tests ($\alpha = 0.05$). The Similar Letters Within the Same Column Show No Substantial Differences. Caption A: Cell Control, B: DMSO Control, C: Combination 1 ($\frac{1}{2}$ IC₅₀ of Turmeric + $\frac{1}{2}$ IC₅₀ Black Rice Bran), D: Combination 2 ($\frac{1}{2}$ IC₅₀ of Turmeric + $\frac{1}{4}$ IC₅₀ Black Rice Bran), and E: Combination 3 ($\frac{1}{2}$ IC₅₀ of Turmeric + $\frac{1}{16}$ IC₅₀ Black Rice Bran)

intensive apoptotic phase. This selectivity is corroborated by the cell control and DMSO control, which demonstrated a high cell viability of 83.27% and 93.67%, respectively, and exhibited necrotic cells of 6.7% and 2.43%, respectively. Furthermore, in a cell culture environment lacking phagocytic clearance machinery, cells undergoing such intensive and synchronized early apoptosis inevitably progress to secondary necrotic fragmentation in vitro (Silva, 2010).

The highly efficient execution kinetics highlight the enhanced multi-target efficacy attained when black rice bran extract was delivered at its optimal therapeutic level in combination with turmeric. Curcuminoids, derived from turmeric, have been extensively documented to induce intrinsic cellular stress. This stress may be mediated by the inhibition of upstream growth regulators, such as EGFR (Ameer et al., 2024), which correlates with the observed reduction in the S phase, a phase frequently associated with DNA damage or incomplete replication (Bertoli et al., 2013).

Simultaneously, the ideal concentration of black rice bran anthocyanins serves as an enhancer (Hartati and Andriyanto,

Table 3. Bioavailability Analysis of Black Rice Bran Compounds According to the Lipinski Rule's

Compound	MW (Dalton)	LogP (<5)	Hydrogen Donor (<5)	Hydrogen Acceptor (<10)	Molar Refractivity (40–130)
Phytosphingosine	317	5.000	3	4	105.994
Monoolein	356	5.422	2	4	115.438
Sinensal	218	4.380	0	1	71.383
(+)-ar-turmerone	216	4.024	0	1	68.649
(8E)-2-amino-8-octadecene-1,3,4-triol	315	2.895	5	4	92.676
1-piperideine	83	1.241	0	1	27.210
3-[(19Z)-15,16-dihydroxy-19-dotriaconten-1-yl]-5-methyl-2(5H)-furanone	312	-0.053	5	6	77.146
Linoleoyl ethanolamide	323	5.054	2	2	109.224
Choline	104	-0.315	1	1	29.985
Oleoyl ethanolamide	325	5.258	2	2	111.558
Betaine	117	-1.557	0	2	27.906
2-aminooctadec-4-yne-1,3-diol	297	3.371	4	3	89.922
Sphinganine	301	5.005	4	2	105.170
(13cis)-2-oxoretinoic acid	314	4.781	1	3	94.152
15-cis-4,4'-diapophytoene	408	10.381	0	0	139.966
2-amino-1,3,4-octadecanetriol	317	4.716	5	3	105.994
2-[(5Z)-5-tetradecenyl]cyclobutanone	264	5.833	0	1	83.332
Baicalein	450	1.765	6	11	99.339
5-oxoETE	318	5.396	1	3	96.430

2022). This formulation is predicted by a molecular docking approach to complementarily target regulators of apoptosis, specifically BCL2- and DR-4. In a theoretical framework, the upstream signals (Caspase-8 and Caspase-9) are presumed to converge, presenting a plausible mechanism for the synchronized activation of downstream executioner enzymes, specifically Caspase-3 and Caspase-7 (Dho et al., 2025; Pebriani et al., 2026). While direct experimental quantification of these internal signaling proteins was not conducted in this study, this literature-supported framework elegantly explains the rapid plasma membrane collapse observed in the phenotypic readout. As the concentration of black rice bran decreases in Combinations 2 and 3, the activation of both pathways appears to diminish, resulting in a lower apoptotic yield.

3.4 Bioavailability and Toxicity Analysis of Turmeric and Black Rice Bran Extracts

Phytochemicals from turmeric and black rice bran extracts were obtained from the study conducted by (Silfarohana et al., 2025). The research identified four compounds in turmeric extract, while black rice bran contained nineteen compounds. The compounds of turmeric included (+)-ar-turmerone, bis-demethoxycurcumin, curcumin, and indole. Meanwhile, black rice bran extracts contained phytosphingosine, monoolein, sinensal, (+)-ar-turmerone, (8E)-2-amino-8-octadecene-1,3,4-

triol, 1-piperideine, 3-[(19Z)-15,16-dihydroxy-19-dotriaconten-1-yl]-5-methyl-2(5H)-furanone, linoleoyl ethanolamide, choline, oleoyl ethanolamide, betaine, 2-aminooctadec-4-yne-1,3-diol, sphinganine, (13cis)-2-oxoretinoic acid, 15-cis-4,4'-diapophytoene, 2-Amino-1,3,4-octadecanetriol, 2-[(5Z)-5-tetradecenyl]cyclobutanone, baicalein, and 5-oxoETE.

The bioavailability assessment was used to determine the drug-likeness of the discovered phytochemicals from both extracts according to Lipinski's Rule of Five. This approach serves as a predictive framework for oral ingestion viability, evaluating molecular weight, lipophilicity, hydrogen-bonding capacity, and molar refractivity rather than adhering to fixed standards (Lipinski, 2004). The screening results revealed that the principal chemical constituents of turmeric (Table 2) and black rice bran extract fulfill these structural criteria (Table 3). The elevated compliance rate designates certain dietary phytochemicals as promising candidates for herbal formulation, indicating advantageous pharmacokinetic characteristics and effective systemic absorption.

The Lipinski-compliant compounds were subjected to *in silico* toxicity profiling to comprehensively assess their safety profiles, concentrating on mutagenicity (AMES test), cardiotoxicity (hERG I/II inhibition), and organ-specific damage. According to Table 4, the computational analysis identified six compounds from black rice bran extract-namely 2-Aminooctadec-

Table 4. Analysis of the Toxicity of Compounds From Black Rice Bran Extract

Compound	AMES Toxicity	hERG I Inhibitor	hERG II Inhibitor	Oral Rat Acute Toxicity (LD50) (mol/kg)	Hepatotoxicity	Minnow Toxicity (log mM)
Phytosphingosine	No	No	No	1.782	No	0.403
Monolein	No	No	No	1.726	No	-0.842
Sinensal	No	No	No	1.567	No	-0.286
(+)-ar-turmerone	No	No	No	1.843	No	0.005
(8E)-2-amino-8-octadecene-1,3,4-triol	No	No	No	1.768	No	0.530
1-piperidineine	No	No	No	2.278	No	2.347
3-[(19Z)-15,16-Dihydroxy-19-dotriaconten-1-yl]-5-methyl-2(5H)-furanone	No	No	No	2.909	No	-4.611
Linoleoyl ethanolamide	No	No	No	1.810	No	-0.672
Choline	No	No	No	1.939	No	2.842
Oleoyl ethanolamide	No	No	No	1.822	No	-0.799
Betaine	No	No	No	1.654	No	2.970
2-aminooctadec-4-yne-1,3-diol	No	No	Yes	1.886	No	-0.025
Sphinganine	No	No	Yes	1.953	No	-0.398
(13cis)-2-oxoretinoic acid	No	No	No	1.845	Yes	-0.064
15-cis-4,4'-diapophytoene	No	No	Yes	1.850	No	-3.358
2-amino-1,3,4-octadecanetriol	No	No	No	1.782	No	0.403
2-[(5Z)-5-tetradecenyl]cyclobutanone	No	No	Yes	1.540	No	-1.125
Baicalein	No	No	Yes	2.444	No	6.149
5-oxoETE	No	No	No	1.515	No	-0.103

4-yne-1,3-diol, sphinganine, 15-cis-4,4'-diapophytoene, 2-[(5Z)-5-tetradecenyl]cyclobutanone, and baicalein-as potential hERG II potassium channel inhibitors, which may be linked to cardiotoxic risks, including arrhythmia (Garrido et al., 2020). Meanwhile, (13cis)-2-Oxoretinoic acid was identified as potentially hepatotoxic. It is crucial to underscore that the bioavailability profiles based on Lipinski's parameters and the toxicological indicators generated by the computational platform remain fundamentally preliminary and purely predictive in nature. These *in silico* simulations function as a preliminary screening framework to assess the potential drug-likeness of the isolated phytochemicals, necessitating subsequent *in vivo* pharmacokinetic validation or definitive toxicological clearance.

Nonetheless, these *in silico* toxicity hazards should be considered judiciously. Although isolated compound simulations indicate structural concerns, the use of complete crude extracts generally involves a complex matrix of co-existing phytochemicals (Ojong et al., 2026). The co-existing antioxidants and cellular protectants frequently produce a synergistic buffering effect (Fisher et al., 2022), counteracting the individual toxicity profiles identified in computational single-ligand models. The

target selectivity is further corroborated by the strong survival of the cell controls observed in *in vitro* studies, indicating that the formulation's destructive capability is highly selective for the malignant HeLa population without demonstrating generalized acute toxicity.

3.5 Molecular Docking

A molecular docking method was utilized to support the multi-target mechanism of phytocompounds extracted from turmeric and black rice bran against cervical cancer targets. The discovered ligands were assessed for their binding affinities to three crucial proteins that regulate cell survival and apoptosis: EGFR (upstream proliferation receptor), BCL-2 (intrinsic anti-apoptotic gatekeeper), and DR-4 (extrinsic death receptor). Methodological validation was performed using native ligand redocking, yielding root-mean-square deviation (RMSD) values below 2 Å (data not shown), thereby confirming the reliability of the active-site coordinates. Table 5 presents the binding affinity values for compounds and their respective protein targets. In addition, Table 6 illustrates the interaction of amino acid residues.

The molecular docking simulations provide structural in-

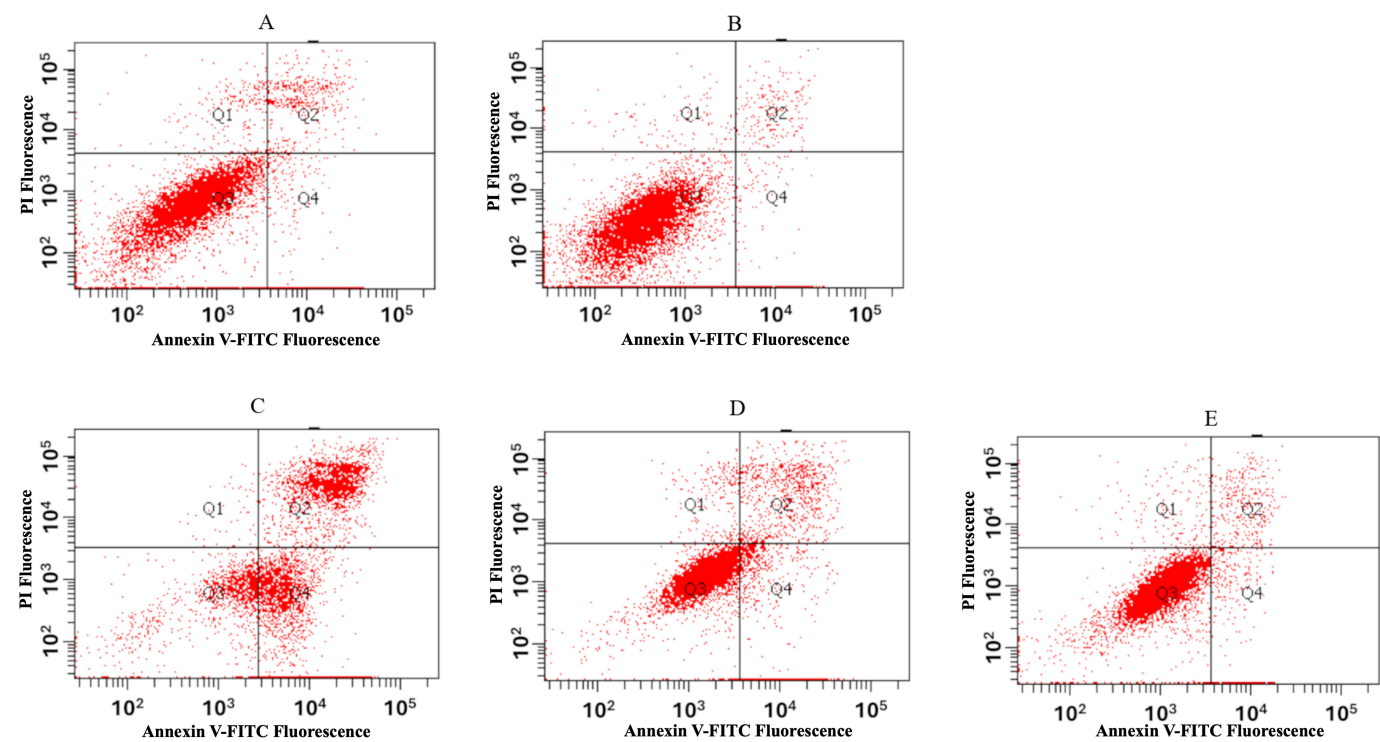


Figure 4. The Apoptosis Distribution was Quantitatively Mapped Across Four Distinct Quadrants Using Annexin V-FITC and PI Signaling; the Lower-Right Quadrant (Q4) Represents Early Apoptotic Cells, the Lower-Left Quadrant (Q3) Denotes Viable Cells, the Upper-Right Quadrant (Q2) Indicates Late Apoptotic Cells, and the Upper-Left Quadrant (Q1) Defines the Necrotic Cell Fraction. Caption A: Cell Control, B: DMSO Control, C: Combination 1 ($\frac{1}{2}$ IC₅₀ of Turmeric + $\frac{1}{2}$ IC₅₀ Black Rice Bran), D: Combination 2 ($\frac{1}{2}$ IC₅₀ of Turmeric + $\frac{1}{4}$ IC₅₀ Black Rice Bran), and E: Combination 3 ($\frac{1}{2}$ IC₅₀ of Turmeric + $\frac{1}{16}$ IC₅₀ Black Rice Bran)

Table 5. The Binding Affinity of Phytocompounds Against EGFR, BCL-2, and DR-4

Extract	Compound/Ligand	Binding Affinity (kcal/mol)		
		EGFR	BCL-2	DR-4
Native Ligand	AQ4	-7.1	–	–
	Venetoclax	–	-9.1	–
	Etoposide	–	–	-7.0
Turmeric	(+)-ar-turmerone	-7.0	-6.6	-4.8
	Bisdemethoxycurcumin	-7.6	-6.9	-5.4
	Curcumin	-7.3	-6.7	-6.1
	Indole	-5.0	-5.0	-4.6
Black Rice Bran	Phytosphingosine	-5.4	-5.3	-4.0
	Monoolein	-4.8	-5.8	-3.9
	Sinensal	-5.4	-6.1	-4.8
	(+)-ar-turmerone	-7.0	-6.6	-4.8
	(8E)-2-amino-8-octadecene-1,3,4-triol	-5.6	-5.1	-4.3
	1-piperidine	-3.5	-3.8	-3.5
	3-[(19Z)-15,16-dihydroxy-19-dotriaconten-1-yl]-5-methyl-2(5H)-furanone	-5.9	-6.1	-4.7
	Choline	-3.4	-3.1	-3.0
	Betaine	-3.4	-3.5	-3.2
	5-oxoETE	-5.6	-6.7	-4.1

Table 6. The Interaction of Turmeric Extract’s Compounds with EGFR

Extract	Compound/Ligand	Binding Affinity (kcal/mol)	Interaction of Amino Acid Residues with EGFR	
			Hydrogen Bond	Hydrophobic Interaction
Turmeric	AQ4	-7.1	Met769 (3.01 Å)	Leu694, Lys721, Leu764, Thr766, Leu768
	(+)-ar-turmerone	-7	Met769 (3.22 Å)	Leu694, Lys721, Leu764, Thr766, Leu768, Leu820
	Bisdemethoxycurcumin	-7.6	Ala719 (2.80 Å), Thr766 (3.14 Å), Met769 (2.91 Å; 2.94 Å)	Leu694, Phe699, Val702, Lys721, Leu764, Ile765, Leu764, Leu768, Leu820, Asp831
	Curcumin	-7.3	Lys721 (3.31 Å), Cys773 (3.24 Å)	Leu820, Lys721, Val702

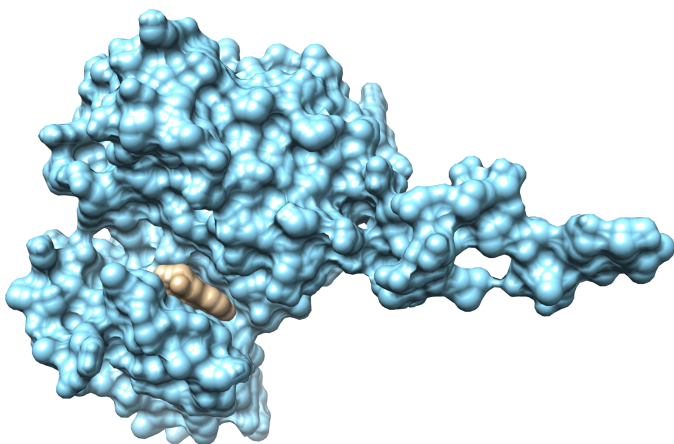


Figure 5. The Pocket Facilitates the Interaction Between Bisdemethoxycurcumin and the EGFR Protein. The Bisdemethoxycurcumin was Colored Cream, and the EGFR Protein was Blue

sight that may help explain the phenotypic alterations observed *in vitro*, supporting a shift from purely observational interpretation toward target-specific hypotheses. The high binding affinity of bisdemethoxycurcumin (−7.6 kcal/mol) (Figure 5) and curcumin (−7.3 kcal/mol) toward the EGFR kinase domain-comparable to the native inhibitor AQ4-indicates a thermodynamically favorable interaction with the receptor binding site. Notably, both bisdemethoxycurcumin and (+)-ar-turmerone (−7.0 kcal/mol) formed stable hydrogen bonds with the key amino acid residue Met769. Mechanistically, Met769 is a highly conserved residue within the ATP-binding hinge region of EGFR, essential for stabilizing the catalytic pocket and facilitating downstream kinase phosphorylation (Yang et al., 2023). Through predicted interaction with Met769, these turmeric-derived phytochemicals may sterically interfere with ligand (ATP) binding, thereby potentially modulating downstream

mitogenic signaling cascades. This proposed interaction is consistent with the *in vitro* cell cycle findings: a reduction in the S-phase population (10.70%) and subsequent accumulation at the G2/M checkpoint were observed under the influence of Combination 1.

Concurrently, the multi-target capability of this formulation is further supported by docking profiles against BCL-2 and DR-4 (Table 5). Within this extract matrix, black rice bran constituents may contribute to additional modulatory interactions; (+)-ar-turmerone showed predicted binding to the hydrophobic groove of the anti-apoptotic BCL-2 (−6.8 kcal/mol), while sinensal engaged the death receptor DR-4 (−4.8 kcal/mol). Biologically, the simultaneous targeting of these proteins probably creates a dual-pathway apoptotic trigger. The structural interaction with BCL-2 potentially disrupts its capacity to sequester pro-apoptotic proteins (e.g., Bax/Bak) (Jan and Chaudhry, 2019). In addition, the engagement with DR-4 mimics death ligand binding, potentially initiating the extrinsic caspase-8 activation cascade (Dho et al., 2025). These integrated computational insights suggest that the combined extracts exert multi-target effects through complementary interactions, rather than through a single dominant pathway.

Nevertheless, this study acknowledges several limitations, primarily the absence of a standard chemotherapeutic positive control in the *in vitro* assays. The lack of a purified drug control perhaps limits a direct comparison of absolute anticancer potency. However, the primary objective of this study was to investigate the potential multi-target anticancer effects of the combined turmeric and black rice bran extracts through integrated *in vitro* and *in silico* approaches. Additionally, while the molecular docking simulations predicted the binding affinities and residue interactions at the active sites, this computational approach remains a static evaluation, and subsequent molecular dynamics (MD) simulations were not performed to assess the long-term stability and conformational changes of the protein-ligand complexes under simulated physiological conditions. Consequently, this work provides a baseline frame-

work demonstrating that the combination may influence cell cycle progression and apoptosis-related processes in HeLa cells. To fully benchmark the therapeutic index, structural stability, and relative efficacy of this herbal combination, both standard chemotherapeutic agents and advanced MD simulations will be integrated into subsequent *in vivo* and molecular validation phases.

4. CONCLUSIONS

This study indicates that the combined formulation of turmeric and black rice bran extracts demonstrates a promising multi-target activity *in vitro*. The most effective preparation, Combination 1, which contained the optimal phytochemical ratio, suppressed HeLa cell proliferation down to approximately 47.88% at 48 hours. This growth inhibition corresponds to a coordinated cellular disruption, beginning with a notable restriction during DNA replication (S phase) that subsequently bottlenecks the cell population into a prominent G2/M phase arrest. Rather than inducing a transient growth suspension, this optimal formulation promotes a shift toward committed cell death kinetics, characterized by a high proportion of early apoptosis (38.57%) and a subsequent transition into secondary necrosis (28.03%). Structurally, these phenotypic outcomes are supported by molecular docking simulations, which suggest that the curcuminoids from turmeric exhibit favorable binding affinities toward EGFR, while the constituents of black rice bran complementarily target BCL-2 and DR-4. In conclusion, these preliminary findings suggest that this dietary combination is a promising, low-dose candidate for further evaluation and warrant rigorous *in vivo* and proteomic validation to elucidate its therapeutic potential in cervical cancer fully.

5. ACKNOWLEDGMENT

The author extends their sincere gratitude to the Indonesia Endowment Fund for Education Agency (LPDP), under the Ministry of Finance, Republic of Indonesia, for the financial support extended to this research endeavor.

REFERENCES

- Akinwumi, I. A. and O. A. Ambali (2026). Exploring Synergistic Effects of Bioactive Compounds and Pharmaceuticals in Therapeutic Applications. *Journal of Holistic Integrative Pharmacy*, **7**; 102–110
- Ameer, S. F., M. Y. Mohamed, Q. A. Elzubair, E. A. M. Sharif, and W. N. Ibrahim (2024). Curcumin as a Novel Therapeutic Candidate for Cancer: Can This Natural Compound Revolutionize Cancer Treatment? *Frontiers in Oncology*, **14**; 1438040
- Bertoli, C., J. M. Skotheim, and R. A. M. De Bruin (2013). Control of Cell Cycle Transcription During G1 and S Phases. *Nature Reviews Molecular Cell Biology*, **14**(8); 518–528
- Chen, J. (2016). The Cell-Cycle Arrest and Apoptotic Functions of p53 in Tumor Initiation and Progression. *Cold Spring Harbor Perspectives in Medicine*, **6**(3); a026104
- Chen, Q. H., L. Dejean, X. Zhang, R. Wang, and G. Chen (2015). The Effects of Curcumin-Based Compounds on Proliferation and Cell Death in Cervical Cancer Cells. *Anticancer Research*, **35**(10); 5293–5298
- Dho, S. H., M. Cho, W. Woo, S. Jeong, and L. K. Kim (2025). Caspases as Master Regulators of Programmed Cell Death: Apoptosis, Pyroptosis and Beyond. *Experimental and Molecular Medicine*, **57**; 1121–1132
- Du, Y., F. Karatekin, W. K. Wang, W. Hong, and G. T. K. Boopathy (2025). Cracking the EGFR Code: Cancer Biology, Resistance Mechanisms, and Future Therapeutic Frontiers. *Pharmacological Reviews*, **77**; 100076
- Fisher, D. R., T. Zheng, D. F. Bielinski, M. E. Kelly, D. S. Cahoon, and B. Shukitt-Hale (2022). Phytochemical Combination Is More Effective Than Individual Components in Reducing Stress Signaling in Rat Hippocampal Neurons and Microglia *In Vitro*. *International Journal of Molecular Sciences*, **23**(20); 12651
- Garrido, A., A. Lepailleur, S. M. Mignani, P. Dallemagne, and C. Rochais (2020). hERG Toxicity Assessment: Useful Guidelines for Drug Design. *European Journal of Medicinal Chemistry*, **195**; 112290
- Hartati, F. K. and Andryanto (2022). Black Rice Extract Induced Apoptosis and Cell Cycle Arrest on Human Cancer Cell Lines. *Current Research in Nutrition and Food Science*, **10**(3); 971–979
- Hu, A., J. J. Huang, J. F. Zhang, W. J. Dai, R. L. Li, Z. Y. Lu, J. L. Duan, J. P. Li, X. P. Chen, J. P. Fan, W. H. Xu, and H. L. Zheng (2017). Curcumin Induces G2/M Cell Cycle Arrest and Apoptosis of Head and Neck Squamous Cell Carcinoma *In Vitro* and *In Vivo* Through ATM/Chk2/p53-Dependent Pathway. *Oncotarget*, **8**(31); 50747–50760
- Huang, Y. P. and H. M. Lai (2016). Bioactive Compounds and Antioxidative Activity of Colored Rice Bran. *Journal of Food and Drug Analysis*, **24**(3); 564–574
- Hyun, J. W. and H. S. Chung (2004). Cyanidin and Malvidin from *Oryza sativa* cv. *Heugjinjubyee* Mediate Cytotoxicity Against Human Monocytic Leukemia Cells by Arrest of G2/M Phase and Induction of Apoptosis. *Journal of Agricultural and Food Chemistry*, **52**(8); 2213–2217
- Ilyas, S. and D. Lee (2025). Exploring the Role of BCL2 Interactome in Cancer: A Protein/Residue Interaction Network Analysis. *Biology*, **14**(3); 261
- Jan, R. and G. E. S. Chaudhry (2019). Understanding Apoptosis and Apoptotic Pathways Targeted Cancer Therapeutics. *Advanced Pharmaceutical Bulletin*, **9**(2); 205–218
- Kania, N., E. Y. Rahman, D. Priyandoko, A. H. N. Sabrina, W. Widowati, R. Azis, A. Annaba, D. S. Hadiprasetyo, and G. Alexandro (2025). The Potential of Ethanol Extract of Pasak Bumi Roots (*Eurycoma longifolia* Jack) as an Anti-Prostate Cancer *In Vitro* Against PC-3 Cells. *Science and Technology Indonesia*, **10**(2); 452–466
- Kurniawan, C., J. W. Siagian, and S. Hutomo (2016). Sitotoksitas Ekstrak Etanolik *Curcuma longa* pada Sel HeLa: Studi *In Vitro*. *Berkala Ilmiah Kedokteran Duta Wacana*, **1**(3);

- 165–171
- Lezaja, A. and M. Altmeyer (2018). Inherited DNA Lesions Determine G1 Duration in the Next Cell Cycle. *Cell Cycle*, **17**(1); 24–32
- Liang, Y., J. Zhao, H. Zou, J. Zhang, and T. Zhang (2021). In Vitro and In Silico Evaluation of EGFR Targeting Activities of Curcumin and Its Derivatives. *Food & Function*, **12**(21); 10667–10675
- Lin, H., C. H. Wu, H. C. Fu, and Y. C. Ou (2025). Recent Advances in Cervical Cancer Treatment: Innovations from Early-Stage to Advanced Disease. *Taiwanese Journal of Obstetrics and Gynecology*, **64**; 608–615
- Lipinski, C. A. (2004). Lead- and Drug-Like Compounds: The Rule-of-Five Revolution. *Drug Discovery Today: Technologies*, **1**(4); 337–341
- Marema, T. P., K. Laka, and Z. Mbita (2026). Medicinal Plants for Overcoming Drug Resistance in Cervical Cancer. *Biology*, **15**(2); 191
- Martinotti, S. and E. Ranzato (2020). Scratch Wound Healing Assay. *Methods in Molecular Biology*, **2109**; 225–229
- Miranda, C. A. N., A. T. B. d. Souza, A. K. M. d. C. Soares, E. Bernardes-Oliveira, H. A. O. Rocha, E. G. Barbosa, T. Guaratini, N. L. Silva, R. N. Cobucci, R. B. Giordani, and J. C. d. O. Crispim (2025). Apoptosis and G2/M Phase Cell Cycle Arrest Induced by Alkaloid Erythraline Isolated from *Erythrina velutina* in SiHa Cervical Cancer Cell. *International Journal of Molecular Sciences*, **26**(10); 4627
- Nair, A. and S. M. Manohar (2021). A Flow Cytometric Journey into Cell Cycle Analysis. *Bioanalysis*, **13**(20); 1627–1644
- Nurani, L. H., F. Azzahra, D. Utami, C. A. Edityaningrum, A. Guntarti, L. M. Irham, I. Daud, A. Khairurrizki, I. L. I. Putra, N. Ismiyati, S. Rofida, and Sugiyanto (2025). Cytotoxic Potential of Combination of *Hibiscus sabdariffa* L. Extract and Cisplatin Against HeLa Cervical Cancer Cell Line: A Study of Antiproliferative Activity and Apoptosis Induction. *Majalah Obat Tradisional*, **30**(2); 121–133
- Oh, S. J., J. Y. Lim, M. K. Son, J. H. Ahn, K. H. Song, H. J. Lee, S. Kim, E. H. Cho, J. Y. Chung, H. Cho, H. Kim, J. H. Kim, J. Park, J. Choi, S. W. Hwang, and T. W. Kim (2023). TRPV1 Inhibition Overcomes Cisplatin Resistance by Blocking Autophagy-Mediated Hyperactivation of EGFR Signaling Pathway. *Nature Communications*, **14**(1); 3329
- Ojong, C., S. A. Besong, and A. N. A. Aryee (2026). Solvent-Based Extraction Recovers Phytochemicals from Medicinal Plants Demonstrating Anticancer and Chemopreventive Potential: A Review. *Molecules*, **31**(7); 1202
- Pebriani, S. A., W. S. Bhagawan, M. E. Prastiyanto, R. M. Rukmana, R. Pratiwi, and A. Suhendi (2026). Anticancer Efficacy of a Combination of *Curcuma longa* L. Rhizome and *Annona muricata* L. Leaf Extracts Against T47D Cells. *Science and Technology Indonesia*, **11**(2); 1–15
- Pratiwi, R., W. A. S. Tunjung, and A. R. Amalia (2015). Black Rice Bran Extracts and Fractions Containing Cyanidin 3-Glucoside and Peonidin 3-Glucoside Induce Apoptosis in Human Cervical Cancer Cells. *Indonesian Journal of Biotechnology*, **20**(1); 69–76
- Rahman, A. T., Rafia, A. Jethro, P. Santoso, V. D. Kharisma, A. A. A. Murtadlo, D. Purnamasari, N. H. Soekamto, A. N. M. Ansori, Kuswati, R. S. Mandeli, K. A. M. S. Ale-dresi, N. F. M. Yusof, V. Jakhmola, M. Rebezov, R. Zainul, K. Dobhal, T. Parashar, M. A. Ghifari, and D. A. P. Sari (2022). In Silico Study of the Potential of Endemic Sumatra Wild Turmeric Rhizomes (*Curcuma sumatrana*: Zingiberaceae) as Anti-Cancer. *Pharmacognosy Journal*, **14**(6); 806–812
- Roberts, S. K., I. Galgadas, D. T. Clarke, L. C. Zanetti-Domingues, F. L. Gervasio, and M. L. Martin-Fernandez (2025). Targeting Mutant EGFR in Non-Small Cell Lung Cancer in the Context of Cell Adaptation and Resistance. *Drug Discovery Today*, **30**; 104407
- Rukmana, R. M., R. Silfarohana, A. D. P. Putra, D. Safrina, D. Susanti, N. R. Wijaya, M. B. S. Adi, M. E. Prastiyanto, Y. A. Cahyaningrum, R. Nurmaulawati, and W. S. Bhagawan (2025). Phytochemical Profile, Antioxidant Activity and Anticancer Activity of Gamma-Irradiated Black Rice Bran (*Oryza sativa* L.) Ethanol Extract: In Vitro and In Silico Study. *Science and Technology Indonesia*, **10**(2); 781–794
- Rukmana, R. M., N. P. Soesilo, Rumiati, and R. Pratiwi (2016). The Effect of Ethanol Extract of Black and White Rice Bran (*Oryza sativa* L.) on Cancer Cells. *Indonesian Journal of Biotechnology*, **21**(1); 63–69
- Rukmana, R. M., N. P. Soesilo, Rumiati, and R. Pratiwi (2017). Chemopreventive Activities of 'Woja Laka' Black Rice Bran Fractions on Liver Carcinoma HepG2 Cells. *Biomedical and Pharmacology Journal*, **10**(4); 1677–1684
- Sachet, M., Y. Y. Liang, and R. Oehler (2017). The Immune Response to Secondary Necrotic Cells. *Apoptosis*, **22**; 1189–1204
- Saeed, M. E. M., R. Yucer, M. Dawood, M. E. F. Hegazy, A. Drif, E. Ooko, O. Kadioglu, E. J. Seo, F. S. Kamounah, S. J. Titinchi, B. Bachmeier, and T. Efferth (2022). In Silico and In Vitro Screening of 50 Curcumin Compounds as EGFR and NF- κ B Inhibitors. *International Journal of Molecular Sciences*, **23**(7); 3966
- Sevindik, M., A. E. Uygün, I. Uysal, and A. E. Sabik (2025). A Comprehensive Review on the Biological Activities, Usage Areas, Chemical and Phenolic Compositions of *Lycium barbarum* Used in Traditional Medicine Practices. *Science and Technology Indonesia*, **10**(1); 173–182
- Silfarohana, R., K. N. Waskita, N. H. Mar'ah, M. S. Rohman, and R. M. Rukmana (2025). Investigating the Potential Synergistic Effects of Turmeric Extract and Black Rice Bran as Cytotoxic Agents Against HeLa Cells. *Asian Pacific Journal of Cancer Prevention*, **26**(3); 1059–1067
- Silva, M. T. (2010). Secondary Necrosis: The Natural Outcome of the Complete Apoptotic Program. *FEBS Letters*, **584**(22); 4491–4499
- Sitarek, P., A. Merecz-Sadowska, T. Kowalczyk, J. Wieceńńska, R. Zajdel, and T. Śliwiński (2020). Potential Synergistic Action of Bioactive Compounds from Plant Extracts Against

- Skin Infecting Microorganisms. *International Journal of Molecular Sciences*, **21**(14); 5105
- Vogler, M., Y. Braun, V. M. Smith, M. A. Westhoff, R. S. Pereira, N. M. Pieper, M. Anders, M. Callens, T. Vervliet, M. Abbas, S. Macip, R. Schmid, G. Bultynck, and M. J. S. Dyer (2025). The BCL2 Family: From Apoptosis Mechanisms to New Advances in Targeted Therapy. *Signal Transduction and Targeted Therapy*, **10**; 1–31
- Wang, J., Q. Wang, D. Lu, D. Wang, R. Feng, K. Wang, R. Moldar, J. Xie, and T. Wen (2017). A Biosystems Approach to Identify the Molecular Signaling Mechanisms of TMEM30A During Tumor Migration. *PLoS ONE*, **12**(6); e0179900
- Yang, H., Z. Zhang, Q. Liu, J. Yu, C. Liu, and W. Lu (2023). Identification of Dual-Target Inhibitors for Epidermal Growth Factor Receptor and AKT: Virtual Screening Based on Structure and Molecular Dynamics Study. *Molecules*, **28**(22); 7607
- Zamrus, S. N. H., M. N. Akhtar, S. K. Yeap, C. K. Quah, W. S. Loh, N. B. Alitheen, S. Zareen, S. N. Tajuddin, Y. Hussin, and S. A. A. Shah (2018). Design, Synthesis and Cytotoxic Effects of Curcuminoids on HeLa, K562, MCF-7 and MDA-MB-231 Cancer Cell Lines. *Chemistry Central Journal*, **12**(1); 1–10
- Zhang, X., L. Zhu, X. Wang, H. Zhang, L. Wang, and L. Xia (2023). Basic Research on Curcumin in Cervical Cancer: Progress and Perspectives. *Biomedicine & Pharmacotherapy*, **162**; 114590
- Zhang, Y., P. Li, J. Rong, Y. Ge, C. Hu, X. Bai, and W. Shi (2020). Small Molecule CDS-3078 Induces G2/M Phase Arrest and Mitochondria-Mediated Apoptosis in HeLa Cells. *Experimental and Therapeutic Medicine*, **20**(6); 1–9