

Cytotoxic Activity of *Chromolaena odorata* Ethyl Acetate Fraction and Its Inhibitory Mechanism Through Cell Cycle Modulation Against WiDr Colon Cancer Cells

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Abstract

Chromolaena odorata has traditionally been used to treat various diseases such as bacterial and fungal infections, external wounds, stomach ulcers, and vomiting blood. Its anticancer activity has been extensively studied. This study aims to prove the cytotoxic effect of *Chromolaena odorata* leaves on WiDr colon cancer cells and its inhibitory mechanism through cell cycle modulation. Dried *Chromolaena odorata* leaves were extracted by maceration using 96% ethanol. They were then triturated with n-hexane and ethyl acetate to obtain n-hexane and ethyl acetate fractions. The ethyl acetate fraction was then screened for phytochemicals using the tube method and tested for cytotoxicity using the MTT assay. The selectivity index (SI) was calculated by comparing the IC₅₀ of WiDr cells to Vero cells. Cell cycle modulation was then observed using flow cytometry. The results showed that the ethyl acetate fraction of *Chromolaena odorata* leaves contained flavonoids, phenolics, and tannins, and was moderately cytotoxic to WiDr cells with an IC₅₀ of 207.69 µg/mL, with an SI of 3.5. The ethyl acetate fraction of *Chromolaena odorata* was able to inhibit the growth of WiDr colon cancer cell division through the G2/M and Sub G1 phases. Thus, it was concluded that the ethyl acetate fraction of *Chromolaena odorata* has great potential to be developed as a candidate source of colon anticancer agents.

Keywords: Cytotoxicity, WiDr, colorectal, *Chromolaena odorata*

1 Introduction

Cancer is a disease characterized by uncontrolled cell growth, invasion of surrounding tissues, and spread (metastasis) to other parts of the body [1]. In cancer, cells lose their control over the cell cycle and homeostasis, allowing them to proliferate continuously and form abnormal tissues [2]. In normal cells, antiproliferative signals work to maintain cell order and tissue homeostasis. Anti-growth signals can block proliferation through two different mechanisms: cells are forced out of the active proliferation phase into the G0 resting phase, or cells are induced to permanently lose their proliferation potential and enter the post-mitotic phase [3].

Cancer cases worldwide continue to increase. In the last five years, there have been 775,120 cases of cancer in Indonesia. Cervical and lung cancer remain the most common types of cancer in Indonesia, followed by breast cancer and colorectal cancer [4]. Given this data, colorectal

cancer has become a cancer that requires the attention of the Indonesian health sector. Colorectal cancer is a malignancy that originates in the large intestine, which consists of the colon and/or rectum. This cancer can occur due to genetic inheritance, genetic mutations, or lesions in the colon caused by infection [5]. Currently, colorectal cancer ranks among the top three most common cancers worldwide, both in terms of incidence and mortality [4]. This is due to changes in modern lifestyles that tend to be unhealthy, such as smoking, alcohol consumption, lack of exercise, or obesity.

Colorectal cancer is usually treated by removing cancerous tissue in the early stages, using chemotherapy for palliative purposes, and using radiotherapy. All of these treatments are considered inadequate due to the side effects they cause, such as drug resistance, systematic toxicity effects, and high treatment costs and decline in quality of life [6]. Given this, the development of

comprehensive therapies to treat cancer is urgently needed to reduce the number of deaths among patients. To this end, the discovery and development of new cancer drugs derived from medicinal plants is essential.

A substance or compound from a medicinal plant can be said to have anticancer activity if it has been proven through various tests, one of which is a cytotoxicity test. Cytotoxicity is the ability of a substance to cause death or damage to cancer cells. Cytotoxicity testing helps identify the effective dose of an agent without causing significant damage to normal cells. This is very important because the success of cancer therapy depends on the agent's ability to kill cancer cells while minimizing side effects on normal tissues and organs. Another advantage of cytotoxicity testing is its ability to provide knowledge about the mechanism of action of agents that work by damaging DNA, disrupting the cell cycle, or through other mechanisms specific to cancer cells. Cell cycle modulation is the process of disrupting or slowing down the normal cell division cycle to stop cell growth. Cancer cells often have abnormal cell cycles and proliferate continuously. Cell cycle modulation mechanisms disrupt the phases of cell division, preventing cancer cells from dividing and causing them to die [7].

Chromolaena odorata (*C. odorata*) is a plant from the Compositae family. People use this plant to treat external wounds, stomach ulcers, and vomiting blood [8]. According to Fitrah et al. [9], *C. odorata* can act as an antioxidant, antibacterial [10], and anticancer agent [11, 12, 13, 14]. This plant contains active compounds such as phenolics, flavonoids, saponins, terpenoids, tannins, and steroids. One of the chemical compounds in this plant is 3', 5-dihydroxy-4', 6,7-trimethoxyflavone, which acts as a strong antiproliferative agent against prostate cancer cells [15], cervical (HeLa) [16], cytotoxic against human stomach cancer cells and human breast cancer cells [12] and Methylripariochromene-A, which is cytotoxic to HeLa cells [14] and the compound sinensetin, which induces autophagy and apoptosis in HepG2 hepatocarcinoma cells [13].

Thus, *C. odorata* is highly potential to be explored for its activity as a colon anticancer agent. From the available literature, no studies have reported the activity and compound content of the ethyl acetate fraction of *C. odorata* as an anti-colon cancer agent. Therefore, this study aims to obtain potent activity at the ethyl acetate fraction level as an anti-colon cancer agent

through phytochemical screening, cytotoxicity testing, and cell cycle modulation.

2 Method

2.1 Preparation, Extraction and Fractionation

500 g of powdered *C. odorata* dry sample was macerated in a glass container using 96% ethanol solvent with a sample to solvent ratio of 1:3. This maceration process was carried out with 3x24 hours of stirring for 3 days. Then the filtrate was taken and the residue was remacerated with new solvent and the filtrate was then evaporated with a rotary evaporator at a temperature of 50°C to form an extract. The crude extract (80 g) was triturated with n-hexane and ethyl acetate to obtain n-hexane and ethyl acetate fractions [17].

2.2 Phytochemical Screening Test [18]

Dissolve 0.1 g of ethyl acetate fraction in 10 ml of CHCl_3 and 4 drops of NaOH, then filter into a test tube and shake. Add H_2SO_4 and shake until two layers form. The upper layer is taken for testing with Mayer's reagent, Wagner's reagent, and Dragendorff's reagent. Sample extracts that are positive for alkaloids with Mayer's reagent will produce a white precipitate, Wagner's reagent will produce a reddish-brown precipitate, and Dragendorff's reagent will produce an orange precipitate.

2.2.1 Phenol

1 ml of the ethyl acetate fraction solution is placed in a test tube, a few drops of hot water and a few drops of FeCl_3 solution are added. A change in the color of the solution to blue-green or blue-black indicates the presence of phenolic compounds.

2.2.2 Flavonoid

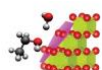
Ethyl acetate fraction is added 2 mL of concentrated hydrochloric acid and 0.2 g of magnesium powder, then shake until it forms an orange-yellow or orange red to purple-red color, which indicates positive flavonoids.

2.2.3 Tannin

Ethyl acetate fraction is added to a 1% gelatin solution containing 10% NaCl. The formation of a white precipitate indicates the presence of tannins.

2.2.4 Saponin

1 ml of ethyl acetate fraction and 1 ml of ethanol are poured into a test tube, then 20 ml of distilled water is added and shaken vigorously for



15 minutes. The formation of stable foam ± 1 cm for 20 minutes indicates the presence of saponins.

2.3 Cytotoxicity and cell cycle assays

2.3.1 Preparation of test solutions

A test stock solution with a concentration of 200,000 $\mu\text{g/mL}$ was prepared by dissolving 20 mg of sample in 100 μL of DMSO, then vortexing until homogeneous. For the *C. odorata* ethyl acetate fraction activity test solution, 2 μL was taken from the stock solution and added to a container containing 798 $\mu\text{L} \approx 800 \mu\text{L}$ of DMEM medium to obtain a concentration of 500 $\mu\text{g/mL}$. Next, dilution was carried out with DMEM medium to obtain test concentrations of 250; 125; 62.5; 31.25; 15.625; $\mu\text{g/mL}$ [19].

2.3.2 Cell Culture

This study used WiDr colon cancer cells. The cells were grown using Dulbecco's Modified Eagle Medium (DMEM) containing 5% Fetal Bovine Serum (FBS); 1% penicillin-streptomycin; and 0.5% amphotericin A, then incubated at 37°C in a 5% CO_2 incubator. Cell harvesting was performed by adding trypsin-EDTA after the cells reached confluence.

2.3.3 Cytotoxicity Assays

The cytotoxicity test of the ethyl acetate fraction of *C. odorata* was performed using the MTT method as described by Pakki et al. [20] and Tanumihardja et al. [21] with slight modifications. A total of 100 μL of cell suspension (104 cells/mL) was added to a 96-well plate and incubated for 24 hours, then 100 μL of extract with various concentrations (15.625–500.00 $\mu\text{g/mL}$) was added. A solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) at a concentration of 0.5 mg/mL was added to each well and incubated again for 4 hours. The reaction was stopped by adding 10% sodium dodecyl sulfate (SDS). The absorbance of each well was measured using a microplate reader at a wavelength of 595 nm. The percentage of inhibition was calculated using Eq. 1.

$$\% \text{ viability} = \frac{(\text{AO treatment} - \text{AO media control})}{(\text{AO cell control} - \text{AO media control})} \times 100\% \quad (\text{Equation 1})$$

Meanwhile, IC_{50} was calculated using

GraphPad 8.0 software.

2.3.4 Selectivity index (SI)

SI is calculated using Eq. 2.

$$\text{SI} = \frac{\text{IC}_{50} \text{ sel Vero}}{\text{IC}_{50} \text{ sel WiDr}} \quad (\text{Equation 2})$$

2.3.5 Cell cycle analysis using flowcytometry

Cell cycle analysis using Propidium Iodide (PI) dye. This dye can be used to analyze the number of DNA sets in each cell. A total of 5×10^5 cells/well were planted in a 6-well plate (for treatment and for cell control). The condition of the cells was observed under a microscope to see the distribution of cells. The cells were then incubated until they returned to normal. Once the cells returned to normal, a series of sample concentrations were prepared for treatment.

Cell cycle induction with a flow cytometer, WiDR cell culture in a 6-well plate was treated with ethyl acetate fractions *C. odorata* at concentrations of $\frac{1}{4} \text{IC}_{50}$, $\frac{1}{2} \text{IC}_{50}$ and IC_{50} . The IC_{50} value was obtained from the linear regression of the relationship between the ethyl acetate fractions concentration and % cell viability. The cells were centrifuged at 1500 rpm for 5 minutes, then the supernatant was discarded, the cell pellet was treated with 500 μL of 70% ethanol preservative drop by drop while shaking, and left at room temperature for 30 minutes. The cells were centrifuged at 500 rpm for 5 minutes, washed with 500 μL of PBS, and treated with propidium iodide (PI) reagent containing 1 mg/mL PI, 10 mg/mL RNase, and 0.1% (v/v) Triton-X 100, then incubated in a dark room for 10 minutes. The cells were transferred to a tube and analyzed with a flow cytometer FACS-Calibur (Fluorescence-Activated Cell Sorter) [19].

3 Result and Discussion

The results of the phytochemical screening test of the ethyl acetate fraction of *C. odorata* are shown in Table 1. These data are qualitative results that indicate the chemical content of the ethyl acetate fraction of *C. odorata*.

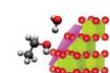


Table 1. Qualitative test results of phytochemical Screening of ethyl acetate fraction of *C. odorata*

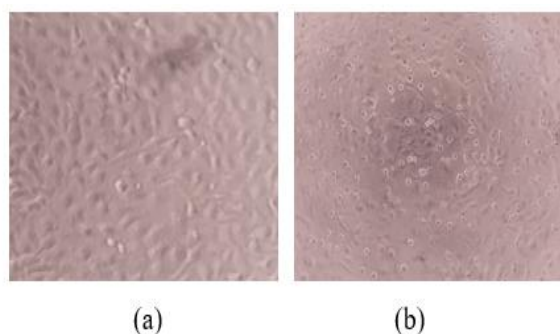
Fraction	Test	Reagent	Results
ethyl acetate	Alkaloids	Mayer	-
		Wagner	
		Dragendroff	
	Flavonoid	HCL pekat + Mg + amil alkohol	+
	Fenol	FeCl ₃ 5%	+
	Tanin	FeCl ₃ 1 %	+
	Saponin	HCl + Water	-

Table 1 shows that the phytochemical screening results of secondary metabolite compounds in the ethyl acetate fraction of *C. odorata* contain positive metabolites of flavonoids, phenolics, and tannins. These results are in line with the identification of ethyl acetate fraction compounds using LC-MS/MS (**Table 2**) which contain phenolic and flavonoid compound components.

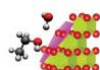
Table 2. LC-MS/MS Results of ethyl acetate fraction of *C. odorata*

No.	Identified Compound	RT (min)	Molecular Formula	[M+H] ⁺ (m/z)
1.	5-Hydroxy-3,6,7 trimethoxy-3',4'-(methylenedioxy) flavone	4.8	C ₁₉ H ₁₆ O ₈	373.09116
	6,7,8-trimethoxy 2H-chromen-2-one	6.6	C ₁₂ H ₁₂ O ₅	237.07550
2.	(2R,3R)-3,7-Dihydroxy-6-methoxyflavanone	7.805	C ₁₆ H ₁₄ O ₅	286.0846
3.	Sakuranetin	7.805	C ₁₆ H ₁₄ O ₅	286.0846
4.	Isosakuranetin	7.805	C ₁₆ H ₁₄ O ₅	286.0846
5.	Naringenin-5-methyl ether	7.805	C ₁₆ H ₁₄ O ₅	286.0846
7.	Averantine	7.14	C ₂₀ H ₂₀ O ₇	373.1290
8.	Guaimisal	7.82	C ₁₆ H ₁₄ O ₅	287.0918
9.	Irganox	9.134	C ₁₈ H ₂₈ O ₃	292.2045

In this study, cytotoxic potential was determined using the IC₅₀ parameter, which is the concentration that causes 50% inhibition of cell growth. The IC₅₀ value was determined from a cytotoxicity test using the MTT method. The ethyl acetate fraction *C. odorata* exhibits concentration-dependent cytotoxic effects, as evidenced by changes in cell morphology and cell viability. Cell morphology changes significantly with increasing concentrations in the ethyl acetate fraction treatment compared to the control cells (**Fig. 1**). Cell viability is determined by the conversion of succinate dehydrogenase enzyme activity to reduce MTT to formazan, which is proportional to cell viability.

**Figure 1.** (a) Live Cell Control WiDr; (b) Treatment with ethyl acetate fraction *C. odorata*

The results showed that the ethyl acetate fraction of *C. odorata* exhibited a concentration-dependent decrease in cell viability, as reflected by decreasing absorbance values with increasing concentrations (**Table 3**). Linear regression analysis yielded the equation $y = -0.0733x + 65.224$ with a determination coefficient ($R^2 = 0.9148$), indicating a strong inverse relationship between concentration and cell survival (**Fig. 2**). The calculated IC₅₀ value of 207.69 µg/mL classified the cytotoxic potency moderate according to the National Cancer Institute criteria [19, 20, 21, 22, 23]. This outcome suggests that the ethyl acetate fraction contains bioactive compounds capable of selectively inhibiting cancer cell proliferation rather than inducing nonspecific toxicity. This is reinforced by the selectivity index (SI) value. **Table 4** show the results that the ethyl acetate fraction of *C. odorata* is selective against WiDr cancer cells with an SI value > 3. The selectivity index value indicates the safety level of an extract/fraction/compound of a drug substance against cancer cells and normal cells, which is calculated by comparing the IC₅₀ value of the extract/fraction/compound against normal cells (Vero cells) and the IC₅₀ against WiDr colorectal cancer cells. The extract/fraction/compound is said to have high selectivity if the selectivity index value is > 3 [19, 20, 21, 22, 23].



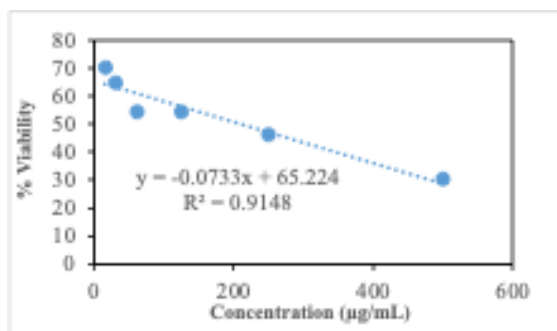


Figure 2. % Viability vs concentration ethyl acetate fraction *C. odorata* against WiDr cancer cell

Table 3. The mean absorbance values and cell viability percentages of WiDr cells of ethyl acetate fraction of *C. odorata* and Doxorubicine

Concentration (µg/mL)	The mean Absorbance	% Viability	IC ₅₀ (µg/mL)	
ethyl acetate fraction				
500	0.28	29.98	31.26	
250	0.37	46.04		
125	0.41	54.20		
62.5	0.41	54.26		
31.25	0.47	64.64		
15.625	0.67	70.08		
Doxorubicin				
10	0.27	23.06		
5	0.28	24.01		
2.5	0.29	26.06		
1.25	0.29	26.51		
0.625	0.30	28.46		
0.3125	0.31	28.86		

Table 4. Selectivity Index (SI) Value

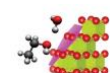
Sample	IC ₅₀		
	WiDr Cell	Vero Cell	SI
Ethyl Acetate Fraction	207.69	727.89	3.5
Doxorubicin	31.26	4.01	0.13

Table 3 shows that treatment of WiDr cancer cells with ethyl acetate fraction of *C. odorata* resulted in decreased cell viability with increasing concentrations. The decrease in cell viability implies interference with key cellular functions, particularly those associated with mitochondrial integrity and metabolic activity. Natural compounds in the ethyl acetate fraction, particularly phenolic and flavonoid metabolites, are known for their ability to disrupt redox homeostasis and trigger apoptosis in tumor cells. Flavonoid metabolites are reported to induce apoptosis through ROS-mediated mitochondrial dysfunction and loss of mitochondrial membrane potential, leading to caspase-3 and caspase-9

activation and modulation of the Bcl-2/Bax ratio [24]. Phenolic constituents together with compounds belonging to the flavonoid class are suggested to disturb the cellular redox balance by increasing reactive oxygen species (ROS) production and reducing endogenous antioxidant defenses, which in turn initiates mitochondrial-dependent apoptotic signaling through a synergistic or additive mechanism that amplifies oxidative stress and promotes programmed cell death [25].

These findings are consistent with previous reviews highlighting that plant-derived phenolic and flavonoid metabolites drive ROS-mediated apoptosis by inducing DNA damage, promoting oxidative imbalance, and disrupting cell cycle progression [26]. Phenolic/Flavonoid compounds (**Table 2**) cause cell cycle inhibition, most often in the (G0/G1) or (G2/M) phases. This prevents cancer cells from continuing to divide into the synthesis (S) or mitosis (M) phases. These compounds modulate regulatory proteins such as cyclins (e.g., cyclin D, cyclin E) and kinase enzyme complexes (CDK2, CDK4) which are commonly overexpressed in WiDr cells. Furthermore, phenolic/flavonoids increase the regulation of tumor suppressor proteins such as p53. Increased levels of this protein act as a checkpoint, preventing cells with damaged DNA from dividing. With prolonged cell cycle arrest, cancer cells will undergo an apoptosis process characterized by an increase in the ratio of pro-apoptotic proteins (such as Bax) compared to anti-apoptotic proteins (such as Bcl-2). The mechanistic framework proposed in those studies supports the idea that natural polyphenols, including flavonoids, modulate oxidative signaling and apoptotic pathways to suppress tumor growth. More specifically, recent evidence in gastrointestinal cancer models indicates that flavonoids exert anti-proliferative effects by downregulating PI3K/Akt and MAPK signaling, activating the p53/p21 axis, and inducing caspase-dependent apoptosis [27].

Furthermore, bioactive flavonoids can regulate cell cycle checkpoints by halting progression at the G₂/M or G₁/S phases and promoting mitochondrial apoptosis through ROS-induced p53 and caspase activation [28]. This mechanistic explanation aligns well with the moderate cytotoxic response and concentration-



dependent inhibition observed in the present study.

To further elucidate the mode of action, cell cycle analysis was conducted using flow cytometry (Table 5). Fig. 3 and Table 5 show that the untreated control cells predominantly occupied the G₀-G₁ phase (54.33%), while treatment with the ethyl acetate fraction at the IC₅₀ concentration reduced the G₀-G₁ population to 36.26% and slightly increased the G₂/M phase to 19.23%. A small but notable increase in the Sub-G₁ fraction from 0.63% to 2.03% was also observed, indicating the onset of apoptosis. This redistribution of cell cycle phases suggests that the fraction interferes with cell cycle progression, causing partial arrest at the G₂/M checkpoint and subsequent apoptotic cell death.

Table 5. Cell Cycle Analysis of Ethyl Acetate Fraction of *Chromolaena odorata*

Concentration	Sub G ₁ (%)	G ₀ -G ₁ (%)	S (%)	G ₂ /M (%)
Cell Control	0.63	54.33	7.13	17.76
IC ₅₀	2.03	36.26	8.06	19.23
½ IC ₅₀	1.36	33.16	7.30	19.03
¼ IC ₅₀	1.36	26.16	6.30	20.06

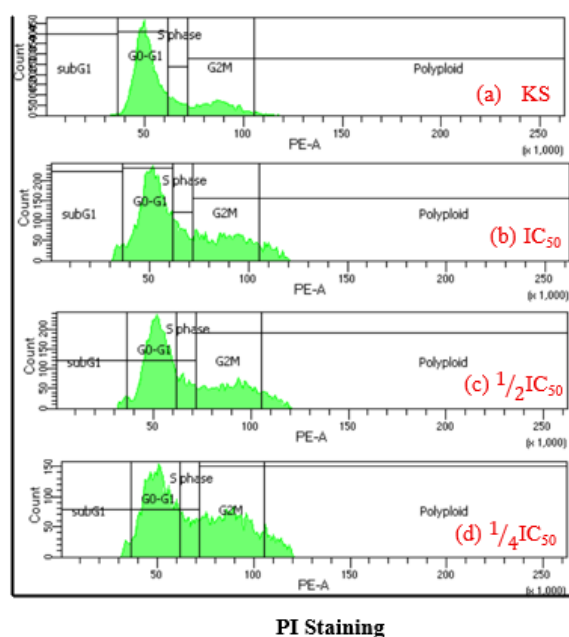


Figure 3. Histogram Profile of Cell Cycle on a) control cells; b) IC₅₀; c) 1/2IC₅₀ and; d) 1/4IC₅₀ concentration treatments of *Chromolaena odorata* Ethyl Acetate Fraction against WiDr Colon Cancer Cell

The progressive increase in the accumulation of cells in the G₂/M phase indicates G₂/M arrest due to DNA damage. This triggers the activation

of the p53 protein, the guardian of the genome, and Cyclin B1. When DNA is damaged, p53 is activated and acts as a transcription factor to express kinase inhibitor proteins such as p21. The p21 complex will arrest the cell cycle, including suppressing the promoter activity for cell division genes. Cyclin B1 is a protein that is essential for the transition from G₂ to the M phase of mitosis by binding to and activating the CDC2 enzyme (CDK1) to form the MPF (Mitosis Promoting Factor) complex. The p53 signaling pathway suppresses the expression of Cyclin B1 mRNA and protein. Because cells lack sufficient Cyclin B1 to trigger the G₂/M transition, cells cannot undergo mitosis and eventually accumulate in the G₂ phase.

This mechanism aligns with reports that flavonoid-rich plant extracts frequently induce G₂/M arrest accompanied by intrinsic apoptosis due to excessive ROS production and mitochondrial impairment. The phenolic and flavonoid constituents of *C. odorata* may thus act as redox modulators, inducing oxidative stress that activates checkpoint kinases and disrupts mitotic progression. As ROS levels exceed the cellular antioxidant capacity, apoptosis is initiated through cytochrome c release, caspase activation, and chromatin condensation, leading to irreversible DNA fragmentation. The integration of these cytostatic (cell cycle arrest) and cytotoxic (apoptotic) mechanisms explains the overall antitumor potential of the ethyl acetate fraction. Collectively, this suggests that *C. odorata* acts through molecular pathways similar to other plant-derived flavonoids that regulate cell cycle control and apoptosis in colon cancer cells [29,30].

4 Conclusion

The ethyl acetate fraction of *Chromolaena odorata* positively contains flavonoids, phenols, and tannins, as well as exhibited moderate cytotoxic activity against WiDr colon cancer cells (IC₅₀ = 207.69 ppm, R² = 0.9148) and induced a concentration-dependent reduction in cell viability. Flow cytometry analysis revealed that the fraction caused redistribution of the cell cycle, characterized by decreased G₀-G₁, mild accumulation in G₂/M, and an increase in the Sub-G₁ population, indicating both cell cycle arrest and apoptosis. Mechanistically, these effects are consistent with ROS-induced mitochondrial dysfunction and activation of checkpoint

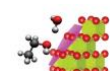
pathways that halt mitosis and promote programmed cell death. The findings highlight the potential of *C. odorata* ethyl acetate fraction as a promising source of natural anticancer agents acting through G₂/M arrest and mitochondrial-mediated apoptosis.

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References

- [1] Nenclares P, Harrington KJ, 2020, The biology of cancer, *Medicine*, 48(2): 67-72.
<https://doi.org/10.1016/j.mpmed.2019.11.001>
- [2] Cooper, G. M., & Adams, K. W. (2023). *The cell: a molecular approach*. Oxford University Press.
- [3] Clark DP, Pazdernik NJ, McGehee MR. Molecular biology. Elsevier; 2018 Nov 2.
- [4] World Health Organization, 2024, International Agency For Research On Cancer, "Global Cancer Observatory," 2024 Februari 8. [Online]. Available: <https://gco.iarc.who.int/Media/Globocan/Factsheets/Populations/900-World-Fact-Sheet.Pdf>. [Accessed 2025 Juni 3].
- [5] Hossain, M. S., Karuniawati, H., Jairoun, A. A., Urbi, Z., Ooi, D. J., John, A., ... & Hadi, M. A. (2022). Colorectal cancer: a review of carcinogenesis, global epidemiology, current challenges, risk factors, preventive and treatment strategies. *Cancers*, 14(7), 1732.
<https://doi.org/10.3390/cancers14071732>
- [6] Fu, B., Wang, N., Tan, H. Y., Li, S., Cheung, F., & Feng, Y. (2018). Multi-component herbal products in the prevention and treatment of chemotherapy-associated toxicity and side effects: a review on experimental and clinical evidences. *Frontiers in Pharmacology*, 9, 1394.
<http://dx.doi.org/10.3389/fphar.2018.01394>
- [7] Holota, M., & Posmyk, M. M. (2025). Nature-inspired strategies in cancer management: The potential of plant extracts in modulating tumour biology. *International Journal of Molecular Sciences*, 26(14), 6894.
<https://doi.org/10.3390/ijms26146894>
- [8] Rusdi, M., Haeria, H., Hamzah, N., Rauf, A., & Amriani, F. (2020). Antiproliferation potential of botto-botto (*Chromolaena odorata* L.) leaves methanol extract fraction against HeLa cell line. *Ad-Dawaa'Journal of Pharmaceutical Sciences*, 3(2).
- [9] Fitrah, M. (2016). Identifikasi Ekstrak Daun Kopasanda (*Chromolaena odorata* Linn) Terhadap Sel Antiproliferasi Tikus Leukemia L1210. *Jurnal Farmasi UIN Alauddin Makassar*, 4(3), 99-105.
- [10] Damayanti, E., & Maryati, M. (2025). Uji antibakteri ekstrak daun kirinyuh (*Chromolaena odorata* L.) dan daun salam (*Syzygium polyanthum*) terhadap *Pseudomonas aeruginosa* dan *Bacillus subtilis*. *Usadha Journal of Pharmacy*, 1-12.
<https://doi.org/10.23917/ujp.v4i1.519>
- [11] Sobrinho, A. C. N., de Moraes, S. M., de Souza, E. B., & dos Santos Fontenelle, R. O. (2017). The genus *Eupatorium* L.(Asteraceae): A review of their antimicrobial activity. *Journal of Medicinal Plants Research*, 11(3), 43-57.
<https://doi.org/10.5897/JMPR2016.6313>
- [12] Razak, N. A., Abu, N., Ho, W. Y., Zambari, N. R., Tan, S. W., Alitheen, N. B., ... & Yeap, S. K. (2019). Cytotoxicity of eupatorin in MCF-7 and MDA-MB-231 human breast cancer cells via cell cycle arrest, anti-angiogenesis and induction of apoptosis. *Scientific reports*, 9(1), 1514.
<https://doi.org/10.1038/s41598-018-37796-w>
- [13] Kim, S. M., Ha, S. E., Lee, H. J., Rampogu, S., Vetrivel, P., Kim, H. H., ... & Kim, G. S. (2020). Sinensetin induces autophagic cell death through p53-related AMPK/mTOR signaling in hepatocellular carcinoma HepG2 cells. *Nutrients*, 12(8), 2462.
<https://doi.org/10.3390/nu12082462>
- [14] Yusuf, H., & Fahriani, M. (2022). Anticancer activity and apoptotic induction of *Chromolaena odorata* Linn leaves extract and fractions on hepatocellular carcinoma cell lines (HepG2). *Jurnal Natural*, 22(1), 57-67.
- [15] Salmela, A. L., Pouwels, J., Kukkonen-Macchi, A., Waris, S., Toivonen, P., Jaakkola, K., ... & Kallio, M. J. (2012). The flavonoid eupatorin inactivates the mitotic checkpoint leading to polyploidy and apoptosis. *Experimental cell research*, 318(5), 578-592.
<https://doi.org/10.1016/j.yexcr.2011.12.014>
- [16] Lee, K., Hyun Lee, D., Jung, Y. J., Shin, S. Y., & Lee, Y. H. (2016). The natural flavone eupatorin induces cell cycle arrest at the G₂/M phase and apoptosis in HeLa cells. *Applied Biological Chemistry*, 59(2), 193-199.
<https://doi.org/10.1007/s13765-016-0160-0>
- [17] Abidin, R. K. S., & Arsianti, A. (2024). Phytochemical screening, antioxidant activity, and cytotoxicity of ethanol, ethyl acetate, and n-



- hexane kluwak (*Pangium edule*) extract on MCF-7 breast cancer cells. *Indonesian Journal of Medical Chemistry and Bioinformatics*, 2(2), 5.
<https://doi.org/10.7454/ijmcb.v2i2.1027>
- [18] Hanani, E. (2017). Analisis fitokimia, Edited By TVD Hardinata and A. Hanif, Penerbit Buku Kedokteran EGC, Jakarta.
- [19] López-Lázaro, M. (2015). A simple and reliable approach for assessing anticancer activity in vitro. *Current Medicinal Chemistry*, 22(11), 1324-1334.
<https://doi.org/10.2174/0929867322666150209150639>
- [20] Radha, A. H. M., & Jawad, K. N. (2021). English: Improvement of the Selectivity Index (SI) and Cytotoxicity Activity of Doxorubicin Drug by Panax ginseng Plant Extract (French: Amélioration de l'Indice de Sélectivité (IS) et de l'Activité, de Cytotoxicité, du Médicament Doxorubicine par l'extrait de Plante Panax ginseng).
- [21] Trujillo, L., Bedoya, J., Cortés, N., Osorio, E. H., Gallego, J. C., Leiva, H., ... & Osorio, E. (2023). Cytotoxic activity of Amaryllidaceae plants against cancer cells: biotechnological, in vitro, and in silico approaches. *Molecules*, 28(6), 2601.
<https://doi.org/10.3390/molecules28062601>
- [22] Mahmood, M. A., Abd, A. H., & Kadhim, E. J. (2024). Assessing the cytotoxicity of phenolic and terpene fractions extracted from Iraqi *Prunus arabica* against AMJ13 and SK-GT-4 human cancer cell lines. *F1000Research*, 12, 433.
<https://doi.org/10.12688/f1000research.131336.3>
- [23] Hasnawati, H., Wahyuono, S., Susidarti, R. A., Santosa, D., & Arfan, A. (2023). A new diterpenoid of Indonesian *Scoparia dulcis* Linn: isolation and cytotoxic activity against MCF-7 and T47D cell lines. *Molecules*, 28(16), 5960.
<https://doi.org/10.3390/molecules28165960>
- [24] Pakki E, Alam G, Syukur R, Muslimin L, 2019, Anticancer Activity of Selected Medicinal Plants Indigenous to Duri Ethnic, *International Journal of Pharmaceutical Research* (09752366), 11.
- [25] Tanumihardja, M., Hastuti, S., Nugroho, J. J., Trilaksana, A. C., Natsir, N., Rovani, C. A., & Muslimin, L. (2020). Viabilities of odontoblast cells following addition of haruan fish in calcium hydroxide. *Open Access Macedonian Journal of Medical Sciences*, 8(D), 58-63.
- [26] Gangopadhyay, A., Chakraborty, S., Jash, S. K., & Gorai, D. (2022). Cytotoxicity of natural flavones and flavonols against different cancer cells. *Journal of the Iranian Chemical Society*, 19(5), 1547-1573.
<https://doi.org/10.1007/s13738-021-02406-6>
- [27] Chen, Y., Yu, D., Zhu, D., Muthusamy, S., Deshpande, M., Kiruthiga, N., ... & Zhu, C. (2025). Exploring alkaloids and flavonoids from natural sources: Emerging natural agents for inhibiting cervical cancer progression through apoptosis induction, anti-inflammatory effects, and oxidative stress reduction. *Pathology-Research and Practice*, 272, 156092.
<https://doi.org/10.1016/j.prp.2025.156092>
- [28] Vidjeyamannane, C., Joy, A., Prakash, K., & Saravanakumar, R. (2025). A comprehensive review on the role of plant-derived bioactive metabolites driving ROS-mediated apoptosis in cancer. *Medical Oncology*, 42(9), 420.
<https://doi.org/10.1007/s12032-025-02985-x>
- [29] Ding, Y., & Yu, Y. (2025). Therapeutic potential of flavonoids in gastrointestinal cancer: Focus on signaling pathways and improvement strategies. *Molecular Medicine Reports*, 31(4), 109.
<https://doi.org/10.3892/mmr.2025.13474>
- [30] Drețcanu, G., Știrbu, I., Leopold, N., Cruceriu, D., Danciu, C., Stănilă, A., ... & Diaconeasa, Z. (2022). Chemical structure, sources and role of bioactive flavonoids in cancer prevention: a review. *Plants*, 11(9), 1117.
<https://doi.org/10.3390/plants11091117>

