

Cytotoxic Activity and Apoptotic Induction of Dichloromethane Fraction *Nauclea subdita* on WiDr Colon Cancer Cell

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Abstract

Colon cancer is one of the most deadly cancers in the world. Conventional treatments such as surgery, radiotherapy and chemotherapy are often limited, especially in metastatic cancer. Therefore, the development of alternative therapies, such as chemoprevention, becomes very important. This study aimed to test the anticancer potential of *bengkak* (*Nauclea subdita*) stem bark against WiDr colon cancer cells through cytotoxic, cell cycle, and Apoptotic assays. The stem bark of *bengkak* was extracted using n-hexane and ethanol, then fractionated using dichloromethane (DCM). The results of the cytotoxic test using the MTT method showed that the DCM pH 3 fraction had the highest cytotoxic potential with an IC_{50} value of 112.81 ± 22.55 μ g/mL against WiDr cells. However, the Selectivity Index (IS) value of the DCM pH 3 fraction showed low selectivity (<2). Cell cycle assay using flow cytometry indicated that DCM pH 3 fraction was able to inhibit cell cycle distribution. Furthermore, Apoptotic test with Annexin V-FITC/PI method showed that DCM fraction pH 3 triggered Apoptotic mechanism in WiDr cancer cells. This study provides preliminary evidence that DCM fraction pH 3 of *bengkak* stem bark has anticancer potential against WiDr colon cancer cells, thus DCM fraction pH 3 of *bengkak* stem bark can be a source of WiDr colon anticancer agent.

Keywords: Apoptotic; Cell Cycle; Cytotoxic; *Nauclea subdita*.

INTRODUCTION

Cancer is one of the main diseases that cause death worldwide with the number of cases increasing every year, both among men and women, so it is a serious concern in the health sector (Agustin, 2019). American Cancer Society (2024) estimates that the number of cancer cases in men reaches 1,029,080, while in women it is around 927,060 (Siegel, 2024). Among various types of cancer, colon and rectal cancer ranks second with 1.926.425 million new cases and 904,019 deaths ((IARC), 2013). In Indonesia, colorectal cancer cases in 2022 reached 35.676 thousand cases while deaths reached 19,255 thousand people (Ferlay, 2020). This fact shows that cancer, especially colon and rectal cancer, is a very serious health problem both at the global and national levels.

The causes of colon cancer are commonly associated with advanced age and a family history of colon cancer. These factors significantly increase the risk of developing colon cancer (Mármol, 2017). Currently, common cancer treatment methods include surgery, radiotherapy and chemotherapy. While surgery and radiotherapy are effective in treating localized and nonmetastatic cancers, they are inefficient for patients

with cancer that has spread. Chemotherapy, which is one of the main treatment methods, has a disadvantage because in addition to destroying cancer cells, it also damages normal cells that have a rapid proliferation rate (Wijaya, 2017). Conventional cancer treatment has limitations, especially in cases of metastasized cancer and severe side effects, so alternative approaches such as chemoprevention are needed.

To overcome the limitations of conventional treatments, cancer experts are developing prevention strategies through lifestyle changes, diet, and chemoprevention. Chemoprevention is the use of natural, biological, or synthetic compounds to inhibit or prevent the change of normal cells into cancer cells. This method is considered one of the most promising approaches in cancer prevention (Puspita, 2016). One of the natural plants that has potential as a chemoprevention is the *bengkak* plant (*Nauclea subdita*). This plant is traditionally used to treat various diseases and is known to contain compounds such as polyphenols, alkaloids, saponins, and flavonoids that have antibacterial and antioxidant activities (Rahmi, 2021). Previous research showed that the dichloromethane extract of *bengkak* stem bark contains monoterpenoid indole alkaloids, namely subditin, which is able to induce Apoptotic and inhibit

the growth of LNCaP AND PC3 cancer cells. Apoptotic is a powerful mechanism to prevent cancer growth because cancer often develops by avoiding this process (Pfeffer, 2018). Although research on the anticancer effect of dichloromethane extract of *bengkal* stem bark has been conducted on LNCaP and PC-3 cancer cells, research on colon cancer, especially on WiDr cancer cells has not been conducted. Therefore, this study aims to determine the cytotoxic mechanism of extracts and fractions of *bengkal* (*Nauclea subdita*) stem bark through evaluation of Apoptotic induction and cell cycle inhibition in WiDr colon cancer.

MATERIALS AND METHODS

Materials

The materials used in this study were WiDr colon cancer cells and Vero normal cells obtained from the WiDr Faculty Parasitology Laboratory, n-hexane, 96% ethanol, dichloromethane (DCM), HNO₃ (Smartlab, USA), NH₃ (Smartlab, USA), universal indicator (Merck, Germany), Sodium Dodecyl Sulfate (SDS) (Merck, USA), Microculture Tetrazolium Technique Assay (MTT) (Sigma, USA), Roswell park memorial institute (RPMI) (Gibco, USA), dimethyl sulfoxide (DMSO) (Merck, Germany), Annexin V-FITC (Gibco, USA), phosphate buffer saline (PBS) (Merck, USA) and propidium iodide (Sigma, USA).

The tools used for this study were separatory funnel (Iwaki), oven (Mettler, Germany), micropipette (Eppendorf, Germany), rotary vacuum evaporator, waterbath (IKA'RV 8, Germany), CO₂ incubator (Mettler), ELISA reader (Benchmark), inverted microscope (Olympus CKX41), 96-well microplate (Iwaki, Japan), conical tube, yellow tip, blue tip, 6 well microplate (Iwaki, Japan), freezer, centrifuge (Thermoscientific), vortex (Thermoscientific, USA), centrifuge (Thermoscientific, USA), CO₂ incubator (Mettler, Germany), laminar air flow (LAF), nitrogen tube, flowcytometer (BD FACSAnto II).

Methods

Sample Preparation and extraction

Samples of *bengkal* (*Nauclea subdita*) stem bark were collected from Ketapang Regency, West Kalimantan in January 2024. The bark was cleaned with running water and then dried. The dried *bengkal* stem bark samples were pulverized using a wood chopping machine. Dry powder of 200 grams of *Nauclea subdita* stem bark was macerated with n-hexane for 3x72 hours and filtered. After filtering the n-hexane extract, the sample was macerated with 96% ethanol for 3x72 hours. The resulting extract was concentrated using a rotary vacuum evaporator at 40°C with a speed of 100 rpm.

Phytochemical screening

Phytochemical screening would be carried out on several tests such as alkaloids, flavonoids, phenolics, triterpenoids, tannins and saponins. The alkaloid test uses Mayer and Wagner reagents (Lantah, 2017), flavonoids use 1 mL of 96% ethanol, sufficient magnesium powder and two drops of HCl 2N (Hidayati, 2013), phenolic test with FeCl₃ 1% (Riwanti, 2019), triterpenoid test uses 2 mL of chloroform and three drops of concentrated H₂SO₄ (Mardhani, 2017), in the tannin test hot distilled water and FeCl₃ 1% (Riwanti, 2019), while the saponin test uses 1 mL of distilled water (Riwanti, 2019).

Fractionation

The liquid ethanol extract was acidified using HNO₃ to pH 3 and then fractionated using dichloromethane (DCM) (1:3) to obtain the DCM pH 3 fraction. Then the DCM pH 3 fraction was concentrated using a rotary vacuum evaporator at 40°C at 100 rpm. Furthermore, the ethanol fraction was alkalized using ammonia (NH₃) to pH 9 and then fractionated with dichloromethanes. The resulting fraction was concentrated using a rotary vacuum evaporator at 40°C at 100 rpm.

Cell Culture

WiDr colon cancer cells and Vero normal cells used in this study were obtained from the Parasitology Laboratory, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia. The cell culture medium used was RPMI-1640 supplemented with 10% FBS (Fetal Bovine Serum), 2% penicillin, and 5% amphotericin. Cells were incubated at 37°C with 5% CO₂ atmosphere in a CO₂ incubator (Masriani, 2014). This test procedure was approved by the Health Research Ethics Commission of Dr. Moewardi Hospital, Solo, Indonesia, with ethical number 1.644/VI/HREC/2024.

Cytotoxic test

WiDr cancer cells and Vero normal cells were harvested from the CO₂ incubator and counted. Cells were diluted with complete medium and added into 96-well microplates with 100 µL of WiDr colon cancer cell suspension at a density of 1x10⁴ cells per well. Cell distribution was examined using an inverted microscope to ensure uniformity of distribution. Then incubate 24 hours in a CO₂ incubator at 37°C with 5% atmospheric humidity. Sample concentration series were prepared for treatment (500, 250, 125, 62.5, 31.25 µg/mL). The media was removed from the incubated microplate, then the concentration series of extracts and fractions of *bengkal* stem bark were added to each well in triplicate and incubated again in a CO₂ incubator at 37°C with 5% atmospheric humidity for 24 hours.

After incubating for 24 hours, 100 µL of MTT reagent (5 mg/mL in PBS MTT concentration) was added to each well and incubated for 4 hours in a CO₂ incubator until formazan crystals formed. After formazan

is formed, add 100 μL of 10% SDS stopper reagent. Next, the plate was placed in the dark at room temperature for 24 hours to dissolve the formazan crystals. The 128 absorbance of each well was read using

an ELISA reader at a wavelength of 595 nm to measure 129 the intensity of the formazan color. The percentage of cell viability was calculated using the formula:

$$\% \text{live cells} = \frac{n(\text{treatment absorbance} - \text{medium absorbance})}{(\text{cell control absorbance} - \text{medium absorbance})} \times 100\%$$

Data in the form of cell viability was used to determine the 50% inhibitory concentration (IC_{50}) based on linear regression between log concentration and percentage of cell viability using the 134 Microsoft Excel program (Eka Nurdiani, 2024).

Test cycle assay

The cell cycle test was performed first on WiDr colon cancer cells with a density of 1×10^4 planted in a 6-well plate and then incubated for 24 hours at 37°C with an atmosphere of 5% CO_2 . Next, the cells were washed with PBS and then treated with DCM fraction pH 3 with two concentration series, namely $\frac{1}{2} \text{IC}_{50}$ and IC_{50} and then incubated. After incubation, cells were harvested using 0.25% EDTA trypsin then added cold PBS and centrifuged for 5 minutes at 1000 rpm. The remaining supernatant was discarded while the pellet formed was vortexed then added 500 μL of Rnase/PI kit solution (Propidium Iodide) and incubated for 15 minutes in the dark at room temperature to avoid fluorescence. The percentage distribution of cells in each phase (G1, S, and G2/M) was analyzed using a FACScalibur flow cytometer. The data obtained from the flow cytometry results will be processed using the flowing program to obtain the percentage of cells in each phase. This test aims to determine how much effect the treatment has on cell cycle inhibition (Lv, 2023).

Apoptotic assay

The Apoptotic test was performed using the Annexin V-FTIV/PI method to detect the apoptotic stage in cells. Cells were pretreated by washing using cold PBS first and then centrifuged, then samples were made in two concentration series, namely $\frac{1}{2} \text{IC}_{50}$, IC_{50} . To each sample, 100 μL buffer and 2 μL Annexin V-FTIC and PI were added. Next, the sample was vortexed until the solution was evenly mixed, then incubated for 10 minutes at room temperature in dark room conditions to avoid fluorescence. After that, the samples were analyzed using flow cytometry to separate cells based on their living and dead status. This analysis was mapped into 4 quadrants to see the cell population, namely the upper left quadrant (Q1, necrosis), upper right quadrant (Q2, late Apoptotic), lower left quadrant (Q3, live cells), lower right quadrant (Q4, early Apoptotic).

RESULTS AND DISCUSSION

Phytochemical screening results

Table 1 showed that n-hexane extracts of *bengkal* stem bark and wood contain alkaloid and triterpenoid compounds, but flavonoids are only found in n-hexane extracts of stem wood.

Table 1. Phytochemical screening results of DCM pH 3 fractions *bengkal* stem bark and wood.

Part of plants	Extract/fraction	Type of testing						
		Alkaloid		flavonoid	Triterpenoid	Fenolik	Tanin	Saponin
		M	W					
<i>Bengkal</i> stem bark	Extract ethanol	-	+	-	+	-	-	-
	Extract n-hexane	+	+	+	-	+	-	-
	Fr.DCM3	++	+	+	-	-	-	-
	Fr.DCM9	+	+	+	-	-	-	-
<i>Bengkal</i> wood	Extract ethanol	+	-	+	+	-	-	-
	Extract n-hexane	+	+	+	-	-	-	-
	Fr.DCM3	+	+	+	+	-	-	+
	Fr.DCM9	+	+	-	-	-	-	-

Description: M = Mayer; W = Wagner

The presence of alkaloid, flavonoid, and phenolic compounds was found in both ethanol extracts of stem bark and stem wood. In the DCM pH 3 fraction of *bengkal* stem bark and wood, the presence of alkaloids

and flavonoids was detected, but the presence of saponins was only detected in the DCM pH 3 fraction of *bengkal* wood. The DCM pH 9 fraction of stem bark and *bengkal* stem wood found the presence of alkaloid

compounds, but the presence of flavonoids was only found in the DCM pH 9 fraction of stem bark. This indicates that all test samples contain alkaloid secondary metabolites. Judging from previous research, there are similarities in the test results with extracts and fractions of *bengkal* stem bark and wood, namely the presence of alkaloid compounds (Wardhani, 2019).

Cytotoxicity of *bengkal* plant againsts WiDr cells

A compound or substance that has the ability to damage normal cells or cancer cells and can even inhibit the growth of malignant tumor cells is referred to as a cytotoxic compound (Tianandari, 2017). MTT (Methyl Thiazolyldiphenyl Tetra bromide) assay is a method used in cytotoxic tests, where the principle of this method is that the mitochondrial dehydrogenase enzyme in the sutochrome of living cells is able to break down the tetrazole ring in the yellow MTT compound, resulting in

purple formazan crystals. The intensity of the purple color produced has a correlation with the number of living cells, otherwise in dead cells, the MTT reagent does not have an effect because the mitochondria do not respire so that the tetrazolium ring does not break, consequently not producing formazan and the solution remains yellow (Doyle, 2000). The parameter used in the cytotoxic test is the IC_{50} value. IC_{50} value is a value that shows how much potential ketoksikan a compound in inhibiting cell proliferation by 50% (Azmy, 2023). The range of IC_{50} values in units of $\mu\text{g/mL}$; among others, <20 (very active); 21-200 (moderate); 201-500 (less active); >500 (inactive) (Darwati, 2024). The results of the cytotoxic test with the treatment of 8 *bengkal* samples against WiDr colon cancer cells and normal Vero cells showed a variety of decreases in cell viability with each increase in the concentration of the sample given, can be seen in Figure 1.

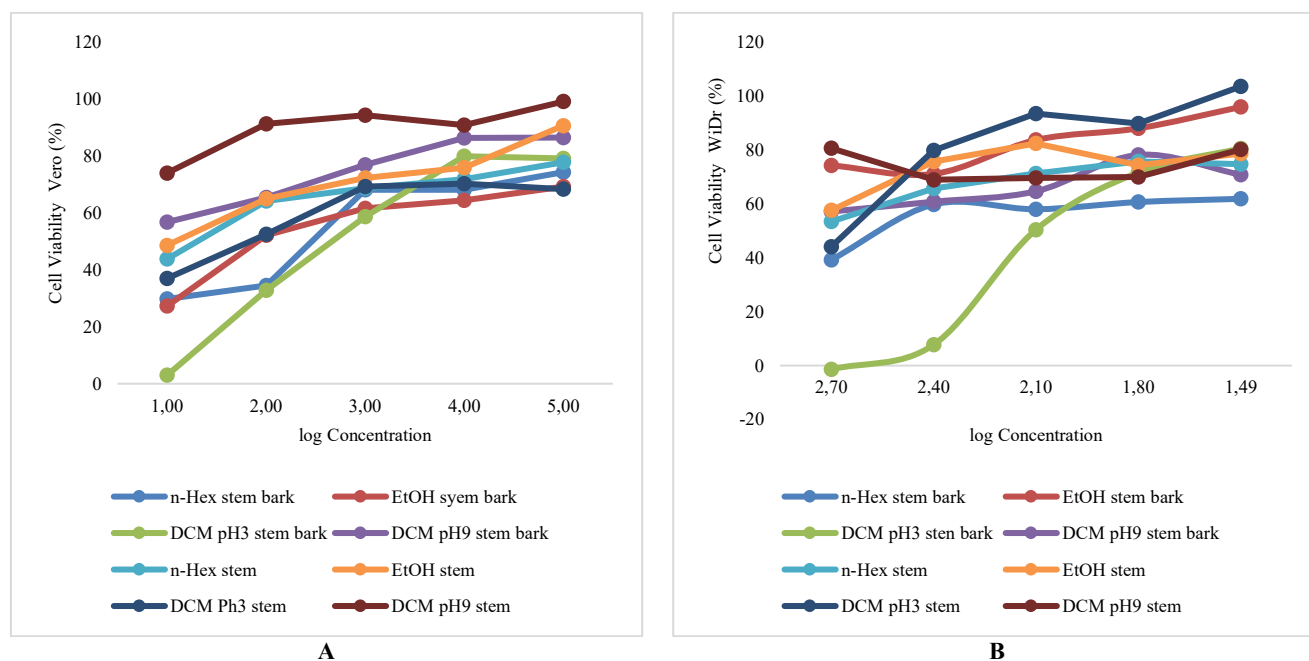


Figure 1. Relationship of Vero cells viability with log concentration againsts various swab samples (A). Relationship of WiDr cell viability with log concentration againsts various swab samples (B)

The results of the analysis on the *bengkal* stem bark sample showed that the lowest IC_{50} value was the DCM pH 3 fraction with an IC_{50} value of $112.81 \pm 22.55 \mu\text{g/mL}$. The selectivity index value in the DCM pH 3 fraction, ethanol extract and n-hexane extract showed $IS < 2$ which means it is not selective, while the DCM pH 9 fraction has an $IS > 2$ value, which means it has good selectivity. The IC_{50} value of DCM pH 3 fraction is lower than other extracts and fractions, which means that this fraction is effective in inhibiting WiDr colon cancer cells. However, the IS value of this fraction is smaller ($IS < 2$) than the DCM pH 9 fraction ($IS > 2$), meaning that

the DCM pH 3 fraction has good anticancer potential, but its selectivity against WiDr colon cancer cells is low. While in the sample of *bengkal* stem wood, the results showed that the IC_{50} in each extract and fraction showed an IC_{50} value of >500 . This indicates that all types of samples are not cytotoxic. In addition, it is also known that the IS value in each type of *bengkal* wood sample is <2 which indicates that the sample is not selective. This indicates that the DCM pH 3 fraction is a sample that has the best IC_{50} compared to other samples, so further investigation needs to be carried out on the DCM pH 3 fraction to determine the IC_{50} .

Table 2. IC₅₀ and selectivity index of extracts and fractions against normal Vero and WiDr cells.

Part of plants	Extract/fraction	IC ₅₀ µg/mL		IS
		WiDr	Vero	
<i>Bengkal</i> bark	Extract ethanol	>500	303.10±124.19	Nd
	Extract <i>n</i> -hexane	>500	186.86±43.63	Nd
	Fr.DCM3	112.81±22.55	146.66±16.52	1.30
	Fr.DCM9	>500	>500	Nd
	Extract ethanol	>500	>500	Nd
<i>Bengkal</i> wood	Extract <i>n</i> -hexane	>500	>500	Nd
	Fr.DCM3	>500	>500	Nd
	Fr.DCM9	>500	303.10±124.19	Nd

Decription:

IS Selectivity index of WiDr colon cancer against Vero normal cells

IS>2 Shows good selectivity

IS<2 Shows no selectivity

SD Standard deviation

Absorbance data that has been obtained can be used to determine the level of cytotoxicity of the sample against WiDr colon cancer cells. In vitro, the selectivity of a compound can be analyzed by comparing the IC₅₀ values of extract and fraction samples against normal Vero cells and WiDr colon cancer cells. The results of this comparison will be presented in the form of the selectivity index (IS) value in Table 2.

Plants that have cytotoxic activity are caused by the content of secondary metabolites. Some plants have been known to have cytotoxic activity, such as plants from the genus *Nauclea*. Isolation of subditine in the dichloromethane extract of *Nauclea subdita* showed inhibitory activity on the growth of prostate cancer cells LNCaP and PC-3 (Liew, 2014). Plants from other genus *Nauclea*, namely *Nauclea latifolia*, show anticancer activity with latifoliamides compounds (Agomuoh, 2013) against MCF-7 breast cancer cells (ME, 2016) and Hep-G2 (Aggrey, 2019). The genus *Nauclea officinalis* also showed cytotoxicity against six human cancer cell lines namely Hep-G2, SK OV3, HeLa, SGC 7901, MCF-7 and KB (Wang, 2024). The *bengkal* stem bark extract is known to contain secondary metabolite compounds such as polyphenols, alkaloids, saponins and flavonoids, while the leaf extract is known to contain secondary metabolite compounds of polyphenols, alkaloids, quinones and flavonoids (Wardhani, 2019). The compound components in the *bengkal* plant can provide pharmacological activity including cytotoxic effects. The cells used in this study are WiDr colon cancer cells. WiDr cells have advantages, namely efficiency reaching 100% in the proliferation process against tumor cells, easy to cultivate, multiplication of WiDr cancer cells is relatively short, namely 15 hours and has a fairly high plating efficiency (Wisnuwardhani, 2021). WiDr cells are often used because these cancer cells have the

advantage of being easy to cultivate and have a shorter doubling time so they are easy to obtain (Yusnita, 1(2), 98-103.).

Cell cycle analysis

Cell cycle tests were conducted on samples that had the highest IC₅₀ values or the highest cytotoxic activity, namely the DCM fraction of pH 3 skin. Cycle analysis used the flow cytometry method to show the percentage of living cells in each cell cycle. The flow cytometry method is able to detect each phase in the cell cycle based on the number of chromosomes in each phase (G1, S, and G2/M). In this method, Propidium iodide (PI) is used because of its ability to interact with DNA, making it easier to determine the distribution of cells in the G1, S, and G2/M phases (Rollando, 2017). Flow cytometry analysis using the flowing program can be seen in figures 2 and 3.

Based on Figure 2, it is known that DCM fraction pH 3 *bengkal* stem bark with concentrations of ½ IC₅₀ and IC₅₀ affects the distribution of WiDr cell cycle. In sub G1 phase, there was an increase in cell accumulation from 1.9% (control) to 2% (½ IC₅₀) and 2.8% (IC₅₀), while in G0-G1 phase, there was a decrease from 64.6% (control) to 60.3 (½ IC₅₀) and 61.9% (IC₅₀). In phase S and G2/M, there was an increase in cell accumulation from 9.4% and 23.4% (control) to 10.8% and 26% (½ IC₅₀) and 11.1% and 23.5 (IC₅₀), respectively. The increase in cell accumulation in the S and G2/M phases indicates that the DCM pH 3 fraction can inhibit the WiDr cell cycle in the S and G2/M phases. The ability of the DCM pH 3 fraction to inhibit the S phase occurred at all concentrations used, while inhibition of the cell cycle in the G2/M phase only occurred at a concentration of ½ IC₅₀.

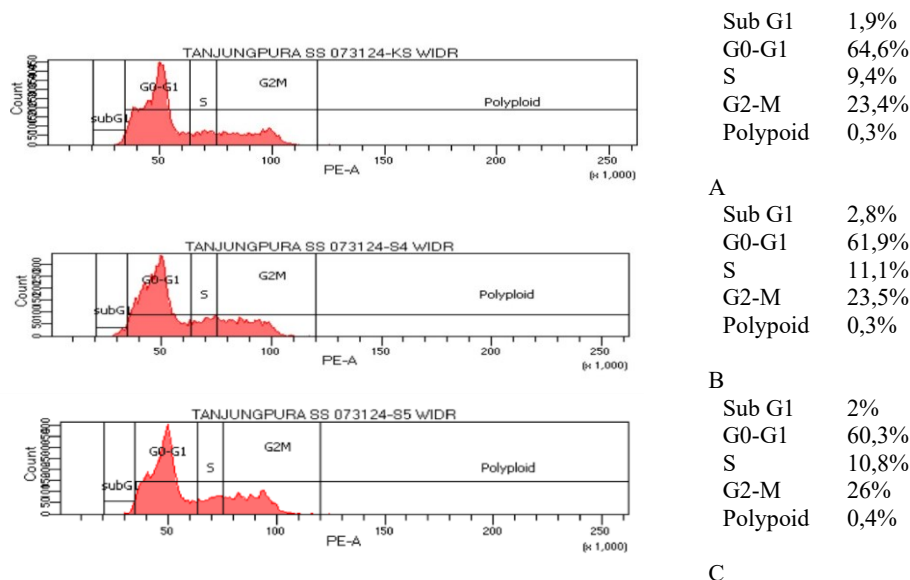


Figure 2. Percentage of cells in each phase of the cycle, A) Cell control; B) IC₅₀; C) ½ IC₅₀.

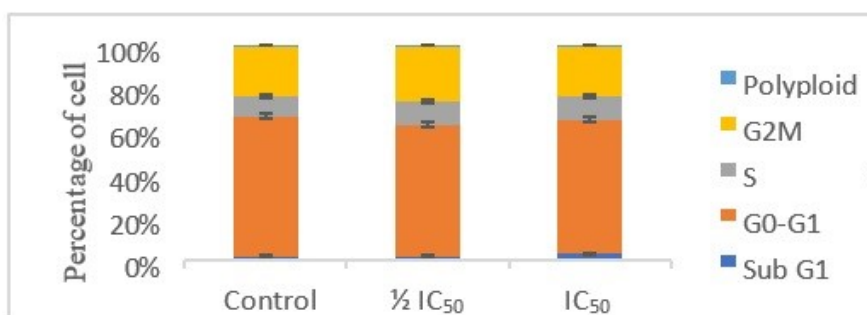


Figure 3. The results of cell cycle analysis by flow cytometry of various concentrations of plant fractions in each phase Sub G1, G0-G1, S, G2/M and Polyploid expressed in histogram form.

In the S phase, cells will duplicate their DNA so that new cells will get a copy of the same DNA or known as DNA replication. Based on the profile in the S phase, it showed that the DCM pH 3 fraction is able to inhibit the cell cycle so that the WiDr cell population increases in the S phase, which means that the DCM pH 3 fraction is able to stop the DNA replication process so that WiDr cells cannot develop and eventually die (T. Novianti, 2020). The G2/M phase is the final stage before cell division (mitosis), where cells in this phase prepare themselves by producing proteins and organelles that will be needed to divide. The G2/M phase profile showed that the sample resulted in an increase in the WiDr cell population in the G2/M phase, which means that WiDr cells cannot continue the cell cycle to the mitotic phase (Vens, 2012). Increased accumulation in sub G1 indicates the occurrence of cell death, tracking the type of cell death is done by flow cytometry method with observation using Annexin-V and PI staining.

Apoptotic analysis with Annexin V-FITC/PI

Apoptotic is programmed cell death that has an important role in maintaining normal tissue homeostatis. Apoptotic mechanism is able to eliminate cells that are no longer needed to maintain physiological balance between surviving cells and cells that die (Hassan, 2014).

Based on the cycle profile, there is an accumulation of cells in the sub G1 phase which indicates that cell death occurs, so the type of cell death that occurs will be tested using the flow cytometry method with observations using Annexin-V and PI staining. Annexin-V is a protein that has a high affinity for phospholipids that are negatively charged due to the presence of Ca²⁺ ions. Calcium contained in Annexin is what will bind Phosphatidylserine (PS) which is on the outside of the plasma membrane that is experiencing Apoptotic. The transfer of phosphatidylserine to the cell surface is what makes Annexin-V bond as a marker of programmed cell death (Apoptotic) (Crowley, 2016). While PI is a red fluorescent dye that will color DNA in a cell that undergoes necrosis and final Apoptotic with membrane damage (Eka Nurdiani, 2024).

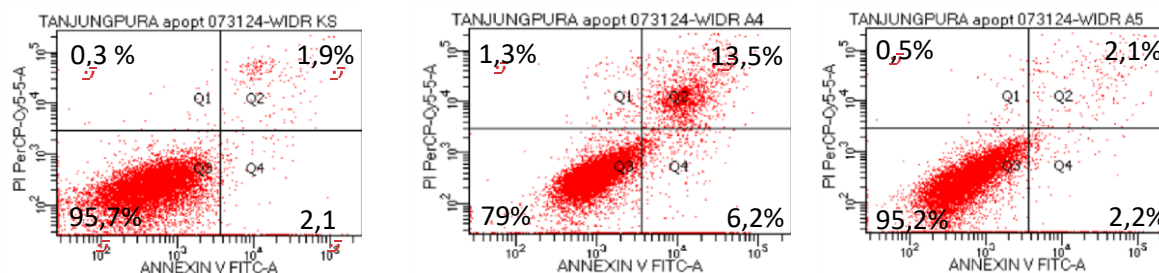


Figure 4. The Apoptotic assay results were grouped into four quadrants, the lower left quadrant (live cell population), the lower right quadrant (early apoptotic cells), the upper right quadrant (late apoptotic cells), and the upper left quadrant (necrosis cells). A) cell control, B) IC₅₀ and C) 1/2 IC₅₀.

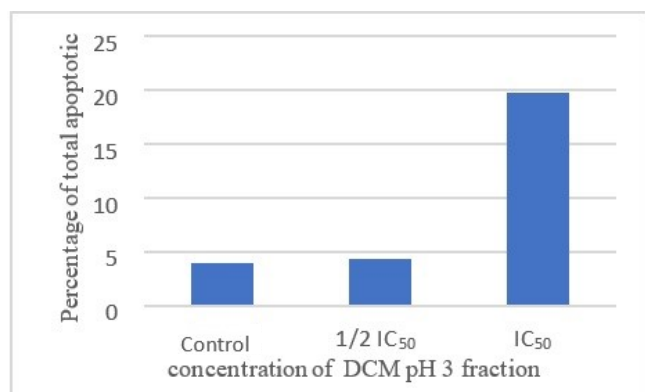


Figure 5. The results of quantitative analysis of Apoptotic of WiDr cell that have been treated by DCM fraction pH 3 of *Nauclea subdita* stem bark at various concentrations of IC₅₀ and 1/2 IC₅₀ for 24 hours with Annexin V-FLOUS/PI analyzed using flow cytometry expressed in diagrams.

The results of Figure 4 and Figure 5, Apoptotic analysis showed that the treatment of DCM fraction pH 3 on WiDr cells affected the number of cells undergoing Apoptotic, both at the early Apoptotic and late Apoptotic stages. In early Apoptotic, the number of cells increased from 2.1% (control) to 2.2% (1/2 IC₅₀) and 6.2% (IC₅₀), while in late Apoptotic, the percentage of cells increased from 1.9% (control) to 2.1% (1/2 IC₅₀) and 13.5% (IC₅₀). Based on the initial Apoptotic and final Apoptotic, total Apoptotic was obtained, which showed that there was a significant increase of 4.2% (control) to 19.7% (IC₅₀) and at a concentration of 1/2 IC₅₀ there was an increase of 4.3%. Meanwhile, necrosis showed that the sample also affected the number of cells from 0.3% (control) to 0.5% (1/2 IC₅₀) and 1.3% (IC₅₀). The results of the analysis showed that the DCM pH 3 fraction of *bengkal* stem bark was able to induce Apoptotic of WiDr colon cancer cells, but along with an increase in the concentration of the sample given based on testing using flow cytometry.

CONCLUSIONS

The study showed that the DCM pH 3 fraction of *bengkal* stem bark (*Nauclea subdita*) has cytotoxic activity against WiDr colon cancer cells with an IC₅₀ value of 112.81 ± 22.55 µg/mL. The cell cycle profile showed that the DCM pH 3 fraction is able to inhibit the WiDr cycle in the synthesis phase so that the DNA replication process stops, besides that there is also

inhibition of WiDr cells in the G2/M phase so that the cells cannot continue to the mitosis phase. In Apoptotic, the DCM pH 3 fraction of swollen stem bark also showed the ability to induce Apoptotic of WiDr colon cancer cells significantly by 4.2% (control) to 19.7% (IC₅₀) and at a concentration of 1/2 IC₅₀ there was an increase of 4.3%.

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