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Activity Anticancer n-hexane Fraction of *Cyperus Rotundus I.* Rhizome to Breast Cancer MCF-7 Cell Line

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Abstract

BACKGROUND: Cancer is one of the causes of morbidity and mortality worldwide. Breast cancer is one of the most common types of cancer in Indonesia. Failures that often occur in the treatment of cancer primarily through chemotherapy, synthetic drugs that have side effects include anemia, alopecia, cardiotoxic and hepatotoxic due to low anti-cancer selectivity and unclear carcinogenesis process. *Cyperus rotundus L.* rhizome is one of the medicinal plants that potential enough to be developed as an anticancer agent.

AIM: The aim of this study was to anticancer activity n-hexane fraction *Cyperus rotundus L.* rhizomes to breast cancer MCF-7 cell line in vitro.

METHODS: *Cyperus rutundus L.* rhizomes powder was extracted ethanol by percolation then fractionated with n-hexane. Phytochemical screening was then carried out. The cytotoxic activity of the n-hexane fraction was determined by observing this extract on MCF-7 cells using the *(3- (4,5-dimethylimidazole-2-il) -2,5-diphenyl tetrazolium bromide)* (MTT). Selectivity index (IS) of normal cells (Vero cells). Cell cycle and apoptosis induction were analyzed by flow cytometry.

RESULTS: The result showed that the fraction n-hexane *Cyperus rutundus L.* rhizome has anticancer activity against breast cancer MCF-7 cells with accumulation cell cycle in the G0-G1 phase and through induction of apoptosis.

CONCLUSION: The n-hexane fraction *Cyperus rotundus L.* rhizome has potent anticancer activity.

Introduction

MCF-7 cells are breast cancer cells that express estrogen receptors (ER+) and originate from pleural effusion breast adenocarcinoma a 69-year-old female patient, blood type O. These cells express estrogen receptor alpha (ER-α), are resistant doxorubicin [1].

The results of GC/MS analysis showed that the main components identified from the *Cyperus rotundus* root essential oils such as α-Cyperone (11.0%), myrtenol (7.9%), caryophyllene oxide (5.4%), β-pinene (5.3%), *Sesquiterpenes* (41.2 %) and *oxygenated monoterpenes* (13.8 %) [2], [3].

Cyperus rotundus L. is known to have cytotoxic effects on some cancer cells, such as leukemia cells K562 and L1210 [4], lymphoma cells of L5178 mice [5], HeLa cells and SiHa cells in cervical cancer [6], Ehrlich's ascites carcinoma breast cancer [7], and KB oral cancer [8].The essential oil potent cytotoxic activities against colon (HCT-116), hepatocellular (Hep G-2) and breast (MCF-7) human cancer cell lines with IC_{50} of 1.06, 1.17 and 2.22 µg/mL, respectively [3].

Therefore this study is to prove whether the rhizome of *Cyperus roundus L.*fractionated with n-hexane has the potential as a breast anticancer performed by cytotoxic testing, selectivity index (IS), induction apoptosis in MCF-7 cells. So, from the test series above the rhizome of *Cyperus rotundus L.* can be used as one of the anticancer drugs.

Material and Methods

The materials that used in this study were MCF-7 (Michigan Cancer Foundation-7) cell and Vero cell culture collection of the Laboratory Parasitology UGM Faculty of Medicine obtained from Prof. Tatsuo Takeya, Japan, DMEM (Dulbecco's Modified Eagle Medium). EDTA Trypsin 0.25%, MTT Reagent (3-(4,5-dimethylimidazole-2-il) -2,5-diphenyl tetrazolium bromide, DMSO (Dimethyl Sulfoxide) Stopper reagent SDS (sodium dodecyl sulfate and Phosphate Buffer Saline). The main ingredients used rhizomes of Cyperus rotundus Linn. obtained from the area named Blang Kolak 1 Village, Bebesen District, Nanggroe Aceh Darussalam Province, Indonesia. The sample was identified at the Herbarium Medanense Institute (MEDA) at the Universitas Sumatera Utara. Authentification number 1941 / MEDA / 2018. The dried powder was percolation used ethanol 96% then fraction n-hexane the obtained percolation was evaporated and freeze-dried [9].

MCF-7 and vero cell 5 x 10^3 / well was implanted into 96-well plate and incubated at 37°C in a 5% CO_2 incubator for 24 hours. Added with various concentrations of the n-hexane fraction Cyperus rotundus L. with 5 variation concentration (500, 250,125, 62,5, and 31,25 µg/mL). Then added 100 mL of MTT reagent concentration (0.5 mg/ml) in DMEM into each well. Then incubated at 37°C again for 3 hours until formazan is formed. The cell condition observation by using microscope. If formazan is formed clearly, a 10% SDS stopper is added in 0.1 N HCl. After that, the plate is again wrapped in paper or aluminum foil and incubated in a dark place overnight. Absorption is read by ELISA reader at λ 595 nm.

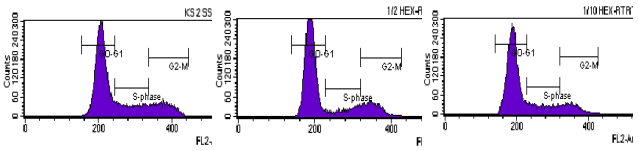
MCF-7 cell line was implanted in six wells, 5 × 10^5 cells/wells. Microplate using coverslips and incubated in 5% CO_2incubator for 24 hours, then for 24 hours. Cycle cell and apoptosis assay were performed two concentrations ($^1/_{10}$ IC_{50} and ½ IC_{50}) n-hexane fraction Cyperus rotundus. Then re-incubated for 24 hours. For cell cycle testing, propidium iodidewhile apoptosis testing added Annexin V and propidium iodide. Then it is measured by a flowcytometer [10].

Cytotoxic activity was expressed by IC_{50}, namely: concentration which caused the death of 50% of the cell population analyzed using SPSS 21 probit analysis with a significance of 0.05 [11].

Results

Based on the calculation above, the results of the IC_{50} n-hexane fraction *Cyperus rotundus* L. was 120.819 µg/mL and selectivity index value 1.831.

The result of accumulation occurs of cycle cells, as can be seen in Figure 1 and Table 1.



(a) (b) (c)

Figure 1: Cycle Cell nHFCR with various concentrations: A) cell control; B) ½ IC_{50}; C) $^1/_{10}$ IC_{50}

Cell cycle analysis is carried out in the cell cycle phase where the accumulation occurs in each treatment. The control group showed MCF-7 cells in the G0-G1, S and G2-M phases respectively 65.48%, 18.31% and 16.65%. After administration of ½ IC_{50}, there was an increase in cell accumulation in the G0-G1 phase to 8.58% while in the S and G2-M phases there was a 7.1% and 1.6% decrease in cell accumulation.

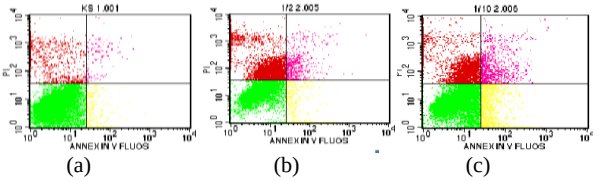
Table 1: Percentage of accumulation in each phase in the cell cycle

Treatment	Concentration	Phase in the cell cycle (%)		
		G0-G1	S	G2-M
Control	-	65.48	28.31	16.65
nHFRT ½ IC_{50}	61.375	74.06	11.21	15.03
nHFRT $^1/_{10}$ IC_{50}	12.27	72.48	15.76	12.13

nHFCR: n-Hexane fraction *Cyperus rotundus.*

The same thing was shown in the treatment with a concentration of $^1/_{10}$ IC_{50} where the G0-G1 phase there was an increase in cell accumulation 7.00% while the phase of S and G2-M phases decreased cell accumulation 2.55% and 4.52%. The change is related to a concentration. However, overall it can be concluded that the cell cycle inhibition mechanism in the G0-G1 phase.

The result of Induction apoptosis can be seen in Figure 2 and Table 2.



(a) (b) (c)

Figure 2: Results of Apoptosis Analysis nHFCR with various concentrations: A) cell control; B) ½ IC_{50}; C) $^1/_{10}$ IC_{50}

In MCF-7 cells given ½ IC_{50}, the percentage of cells experiencing initial apoptosis (2.14%) was seen; The $^1/_{10}$ IC_{50} (7.90%) and compared the control (1.64%). Seen the $^1/_{10}$ IC_{50} increases of cells undergoing initial apoptosis compared to controls. The percentage of cells undergoing final apoptosis (initial necrosis) also increased in the administration of ½ IC_{50} (2.93%).

Table 2: The result of apoptosis analysis for cell MCF-7

Treatment	Concentrate µg/mL	%			
		LL	LR	UR	UL
Control	-	90.25	1.64	0.62	7.49
nHFCR½ IC_{50}	61.375	70.34	2.14	2.93	24.61
nHFCR$^{1}/_{10}$ IC_{50}	12.275	68.06	7.09	4.79	19.25

Where: Bottom left (LL) = living cell; Bottom right (LR) = initial apoptotic cells; Upper right (UR) = late apoptotic cells and initial necrosis; Upper left (UL) = cell necrosis.

The $^{1}/_{10}$ IC_{50} (4.79%) was compared with the control (0.62%). Cells that experienced late necrosis in MCF-7 cells were given ½ IC_{50} (24.61%), $^{1}/_{10}$ IC_{50} (19.25%) shows that the percentage of necrosis increased compared to controls (7.49%).

Discussion

N-hexane fraction *Cyperus rotundus* L. active as an anticancer because an extract is considered active if it has an IC_{50} value of ≤ 100 µg/mL [12]. However, an IC_{50} extract value of 100-500 µg/mL can still be developed as an anticancer with a moderate classification [13]. It can be said that the n-hexane fraction has cytotoxic activity against MCF-7 breast cancer cells and affects normal cell growth (Vero cells).

Inhibition of the G0-G1 cycle allows apoptosis to occur, so there is no activation of CDK4 and CDK6 which results in inhibition of phosphorylation of pRb (protein retinoblastoma), unphosphorylated Rb binds to the transcription factor E2F binds DNA and inhibits gene transcription whose products are required for the phase S cell cycle so that cells are held in phase G1 or G1 arrest occurs [14]. The cessation of the cell cycle in the G0-G1 phase gives the cell a chance to repair damaged DNA and provide an opportunity for damaged cells to be recognized and then proceed with the apoptosis process. Check point control is very important to maintain genomic stability. The error will pass the cell to breed even though there is DNA damage, incomplete replication, the chromosome is not completely separated it will produce genetic damage. This is called cancer. Therefore, the process of cell cycle regulation can play a role in cancer prevention [15].

The n-hexane fraction *Cyperus rotundus L.* shows positive activity in apoptosis by flow cytometry method using Annexin V. The principle of Annexin V labeling is staining on phosphatidylserines (PS) found on the outer membrane of the cell. Early apoptotic cells express PS on the outer plasma membrane. PS can be colored by annexin V label. Cells that undergo final apoptosis and cell necrosis will lose their cell membrane integrity and are permeable to annexin V dyes. The working mechanism of the n-hexane fraction is likely to be in the apoptotic phase (initial necrosis). The potential for n-hexane fraction of the rhizomes in stimulating cell apoptosis is probably caused by steroid terpenoids compounds n-hexane fraction. Terpenoids is compounds that have high anticancer activity that was tested through the ability to block nuclear factor-kappa B, induces apoptosis, activates transcription and angiogenesis, which can be useful in the treatment of various types of cancer [16], [17].

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