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INHIBITORY ACTIVITY OF ALKALOID OF EXTRACT ETHANOL RANTI HITAM (*Solanum blumei Nees ex Blume)* FRUIT ON LEUKIMIA L1210 CANCER CELLS GROWTH

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ABSTRACT

Plants "Ranti Hitam", *Solanum blumei* Nees ex Blume (Solanaceae) were found in Karo and Dairi, North Sumatra, Indonesia. Traditionally ranti hitam used as drugs, such as pain medications, fever, abdominal pain, ear pain, anti-inflammatory and so on. Antioxidants and toxicity test results show that the compound $C_{39}H_{64}NO_{11}$ alkaloid isolated from the ethanol extract of fruit ranti hitam potentially bioactive. This study aims to determine the potential anticancer alkaloid $C_{39}H_{64}NO_{11}$ compound from ethanol extract of fruit ranti hitam by measuring the inhibitory activity of these compounds against L1210 leukemia cancer cell growth *in vitro* by microscopic counting method (Hemocytometer). The results showed that the $C_{39}H_{64}NO_{11}$ alkaloid of the ethanol extract of fruit ranti hitam potentially bioactive anticancer against leukemia cells in L1210 with a value inhibisy concentration 50 (IC_{50}) of 1.2738 µg/mL < 4.0 µg/mL. As a positive control used Doxorubicin with IC_{50} values of 0.1540 µg/mL .

Key words: Ranti hitam (*Solanum blumei* Nees ex Blume), inhibition activity, cancer cell Leukemia 1210, Indonesian medicinal plants .

INTRODUCTION

Based on data from the World Health Organization (WHO) in 2004 , cancer is the second biggest cause of death in developing countries after cardiovascular disease (Anonymous , 2004) . The rate of death caused by cancer tends to increase, estimated of about 9.0 million deaths in 2015, increased to approximately 11.4 million deaths in 2030 (Saiz-Urra *et al*., 2009) . Search an alternative material based anticancer natural materials need to be developed because the material is needed to treat cancer patients continues to increase and to reduce the cost of cancer treatment is relatively very expensive and overcome the side effects of synthetic drug use. Knowledge of medicinal plants should be developed, especially to harness the potential of Indonesia's natural resources are rich with flora. One of the types of plants that are found in the Dairi and Karo, especially Kuta Nangka village , sub-district Tanah Pinem is ranti hitam. The results of determination of plants by the Field Botany Biology- LIPI Research Center, Bogor in March 2013, ranti hitam is kind of *Solanum blumei* Nees ex Blume and including the Solanaceae family. Traditionally ranti hitam used as drugs, such as pain

medications ,fever, abdominal pain, ear pain, anti-inflammatory and so on .Plants generally contain active compounds in the form of secondary metabolites such as alkaloids ,flavonoids,steroids ,triterpenoids, coumarins and others who are potential components of medicinal plants.

The results of the screening study of secondary metabolites , the ethyl acetate extract of the leaves and fruits of *Solanum blumei* Ness ex Blume local, there are alkaloids, steroids, flavonoids , the ethanol extract contained alkaloids , flavonoids , phenols , saponins and tannins slightly, whereas the *n*- hexane extract only contains secondary metabolites, steroids and alkaloids bit . The highest yield of extraction contained in the ethanol extract of the leaves and fruit extracts compared with *n*-hexane and ethyl acetate (Simorangkir *et al* , 2013a). Secondary metabolites contained in the plant *Solanum blumei* Ness ex Blume become potential as a medicinal plant. Test results antioxidants (Simorangkir *et al* , 2013b) and toxicity BSLT (Simorangkir et al , 2014) showed that the ethanol extract of ranti hitam fruit potentially bioactive. Results fractionation ethanol extract of ranti hitam derived alkaloid $C_{39}H_{64}NO_{11}$ potentially bioactive antioxidants and toxicity test based BSLT (Simorangkir , 2014). Based on the potential of bioactive alkaloids $C_{39}H_{64}NO_{11}$ ranti hitam fruit need to be developed as an alternative to anti-cancer drugs by testing the inhibitory activity of alkaloid compounds $C_{39}H_{64}NO_{11}$ ranti hitam against L1210 leukemia cancer cell growth *in vitro*. One way in determining the preliminary test compounds are useful as anti-cancer test inhibitory effect on the growth of L1210 leukemia cells. L1210 leukemia cells are the target of anti-cancer activity of this experiment is one strain mouse leukemia cells were routinely been used to test the anti-cancer compounds, both *in vitro* and *in vivo* using rats. L1210 leukemia cells obtained from The Institute of Physical and Chemical Research Japan (RIKEN). L1210 leukemia cells were then suspended in RPMI -1640 media formula and contains a solution of bovine calf serum. Since the 1955-1975 US National Cancer Institute (NCI, National Cancer Institute) using the L1210 cell line for the initial screening of anti-cancer substances . Substances that are active against L1210 cell lines were tested *in vivo* in mice inoculated with tumor, prior to clinical trials (National Cancer Institute, 2008) .

This study aims to determine the anti-cancer potential of alkaloids $C_{39}H_{64}NO_{11}$ of ethanol extract of fruit ranti hitam by measuring the inhibitory activity of these compounds against L1210 leukemia cancer cell growth *in vitro* .

METHODOLOGY

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Place Research. This research was conducted at the Laboratory of Health Materials PATIR -BATAN Jakarta and Natural Products Chemistry Laboratory Center of Biotechnology LIPI Cibinong.

Materials and Equipment. The materials used are sampled $C_{39}H_{64}NO_{11}$ alkaloid isolated from the ethanol extract of ranti hitam fruit (Simorangkir, 2014), L1210 leukemia cells taken from mice induced in female mice metilklorantren strain DBA /2 which was originally obtained from The Institute of Physical and Chemical Research Japan , RIKEN (obtained from the Laboratory of Health Materials PATIR - BATAN Jakarta), the culture medium Rosewell Park Memorial Institute 1640 (RPMI -1640, Gibco), NaHCO3, Fetal Calf Serum (FCS , Mycoplex $^{(R)}$), doxorubicin (positive control), tryphan blue (Merck) and methanol pa.

The tool used was a 40 mL tissue culture flask (Nunclon), tissue Culture Plate 96 wells (Nunclon), Laminari Air Flow Biological Safety Cabinet (Forma Scientific) , with a flow cell incubator 95 % oxygen and 5 % CO2 (Forma Scientific), tank liquid nitogen (JR Thermolyne Locator), oven , autoclave, 1.5 mL tubes (EppendorfTM), 2 mL sterile serological pipette (Falcon), micropipette (EppendorfTM), microscopy (Nikon TMS) and a hemocytometer (Neubauer haemocytometer impoved, Superior Marienfeld).

Test Cell Growth Inhibition Against Leukemia L1210. *Making the Media*. The manufacturing is done by using RPMI -1640 weighing 10.4 g containing L- glutamine was dissolved in 1 L of sterile water (A) . Then 1.3 g of $NaHCO_3$ dissolved in 50 ml of sterile water (solution B). A total of 25 ml of solution B was added into 475 ml of solution A, then obtained 500 ml of medium (C). For test purposes, 15 ml bovine calf serum was added into 85 ml of solution C. All work is done in a sterile room. L1210 leukemia cells suspended in medium containing calf bovine serum has been bringing the total number of cells is about 2 x 10^5 cells /mL . L1210 leukemia cells used in this study were obtained from The Isntitute of Physical and Chemical Research of Japan (RIKEN).

Inhibitory Power Test. Test of inhibition of L1210 leukemia cell growth of alkaloid compounds $C_{39}H_{64}NO_{11}$ fruit ranti hitam done with dose variation of 0.5 ; 1.0 ; 2.0 ; 4.0 and 8.0 µg / mL of methanol . As a positive control used Doxorubicin dose variation of 0.08 ; 0.16 and 0.31 µg / mL . As a negative control is methanol (solvent sample). Media who had conceived L1210 leukemia cell suspension (2 x 10^5 cells /ml) and samples $C_{39}H_{64}NO_{11}$ alkaloid compounds incorporated into the tissue 's multi- well plate culture so that the total volume of 1 mL in each of the wells. As a negative control used 10µL of methanol was added 990 µL of cell suspension . Experiments conducted duplo, then cell suspension that has been filled samples were incubated for 48 h at 37°C in a 5 % CO_2 incubator. Cell count was performed using a Neubauer haemocytometer improved. To differentiate between living cells with the cell death

prior to the count, 90 µL of the suspension incorporated into the holdings of the cluster plate (96 wells) and plus 10µL solution of 1 % tryphan blue and homogenized. A total of 10µL solution was poured into improved Neubauer haemocytometer. After that the number of surviving cells counted under a microscope. Living cells appear as transparent spheres with a blue dot in the center circle the cell nucleus, whereas dead cells appear as dark blue patches irregularly shaped (Suffines and Pezzuto , 1991) .

The percentage inhibition of the test substance on the growth of L1210 leukemia cells was calculated as % inhibition = (1 - A /B) x 100 %.

A : number of live cells in a medium containing the test substances

B : number of live cells in a medium containing no test substance (control) .

Furthermore, inhibition percentage data were plotted to probit table to obtain the value of probit . Then graphed between log concentration x) and probit (y) in order to obtain the linear regression equation y = a + bx. By entering the value of y = 5 (probit of 50 %), the obtained values of x (log concentration), IC50 value by converting the value of the log concentration to form an anti logs . IC_{50} is the concentration of the test substance that can inhibit cell proliferation by 50 % after an incubation period of 48 hours Ministry of Health, Indonesia Pharmacopoeia, 1995). Activities crystal (isolates) said to be active as an anti-cancer when IC_{50} value ≤ 4 µg / mL.

RESULTS AND DISCUSSION

The test results from the inhibition of alkaloid compounds $C_{39}H_{64}NO_{11}$ ranti hitam fruit and Doxorubicin as a positive control against L1210 leukemia cell growth are presented in Table 1.

Table 1. Test Results Inhibition of Alkaloid Compounds $C_{39}H_{64}NO_{11}$ Ranti Hitam Fruit On L1210 Leukemia Cell Growth

No.	Test material	Dose (µg/mL)	Inhibition (%)	IC_{50} (µg/mL)
1.	Alkaloids $C_{39}H_{64}NO_{11}$ (fruit ranti hitam)	0,50	36,21	1,2738
		1,0	46,5	
		2,0	56,90	
		4,0	67,24	
		8,0	74,14	
2.	Doxorubicin (positive control)	0,08	37,93	0,1540
		0,16	51,72	
		0,32	62,07	

Furthermore, inhibition percentage data were plotted to probit table to obtain the value of probit . Then graphed between log concentration (x) and probit (y) in order to obtain the linear regression equation y = a + bx (Figure 1 and 2).

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By entering the value of y = 5 (probit of 50 %), the obtained values of x (log concentration), IC_{50} values by converting the value of the log concentration to form an anti logs. IC_{50} is the concentration of the test substance that can inhibit cell proliferation by 50 % after an incubation period of 48 hours (Table 2).

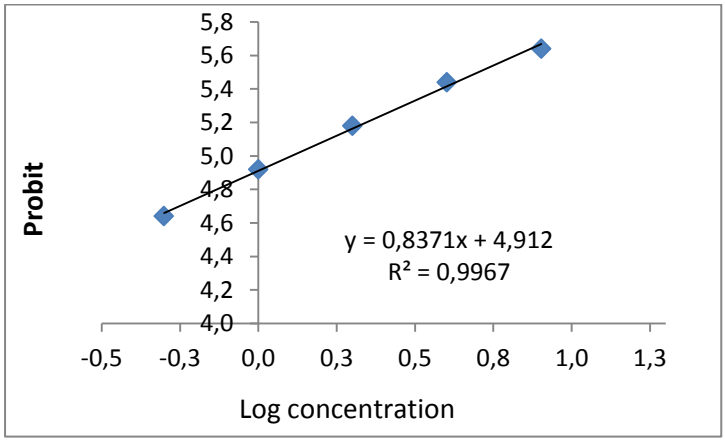

Figure 1. Relationship Graph Alkaloid $C_{39}H_{64}NO_{11}$Log Concentration (x) and Probit (y)

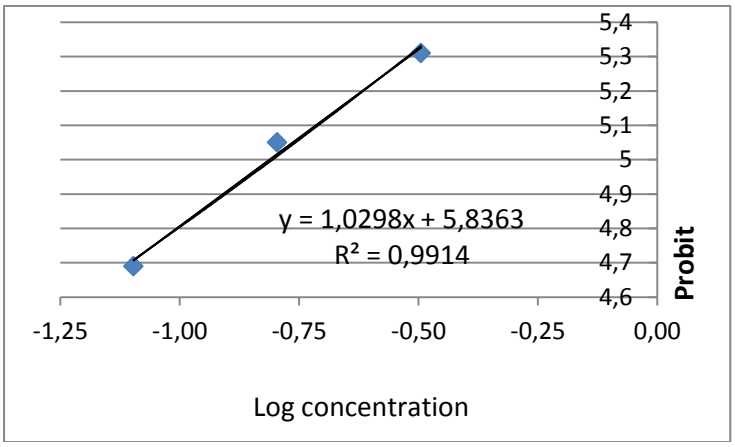

Figure 2. Graph Relations Doxorubicin Log Concentration (x) and Probit (y)

Table 2. Conversion of Alkaloid Compounds IC_{50} Value $C_{39}H_{64}NO_{11}$ and Doxorubicin

No.	Test material	Conversion value	µg/mL
1.	Alkaloids compounds $C_{39}H_{64}NO_{11}$ (fruit ranti hitam)	r	0,9980
		Log IC_{50}	0,1051
		IC_{50}	1,2738
2.	Doxorubicin (positive control)	r	0,9955
		Log IC_{50}	-0,8124
		IC_{50}	0,1540

Alkaloid compound $C_{39}H_{64}NO_{11}$ IC_{50} of 1.2738 (µg / mL) showed concentrations of alkaloid compounds $C_{39}H_{64}NO_{11}$ 1.2738 (µg / mL) can inhibit the proliferation of L1210 leukemia cells by 50 % after an incubation period of 48 hours. Determining whether or not a substance developed as an anti-cancer drug based on the nature of the toxicity. NCI has set criteria based

activity inhibisy value Concentration 50 (IC_{50}) is the concentration required to inhibit cell growth by 50%. A substance called cytotoxic effect when the activity of the test cell has a value of IC_{50} < 20 ug/ml for an extract , and IC_{50} < 4 µg /mL for pure compounds (Suffnes and Pezzuto , 1991) .

The results showed that the compound $C_{39}H_{64}NO_{11}$ alkaloid isolated from the ethanol extract of fruit ranti hitam potentially bioactive anticancer against leukemia cells in 1210 with a value inhibisy concentration 50 (IC_{50}) of 1.2738 ppm< 4.0 ppm. As a positive control used Doxorubicin with IC_{50} value of 0.1540 ppm. Since the 1955-1975 US National Cancer Institute (NCI, National Cancer Institute) using the L1210 cell line for the initial screening of anti-cancer substances. Substances that are active against L1210 cell lines were tested *in vivo* in mice inoculated with tumor, prior to clinical trials (National Cancer Institute , 2008).

CONCLUSIONS AND RECOMMENDATIONS

$C_{39}H_{64}NO_{11}$ alkaloid compounds were isolated from the ethanol extract of ranti hitam fruit *(Solanum blumei* Ness ex Blume) has a value of 50 % inhibitory concentration (IC_{50}) below 4.0 µg / mL is equal to 1.2738 µg /mL against L1210 leukemia cell lines *in vitro* after an incubation period of 48 hours, so that potential as anticancer.

The results of the initial screening test inhibition of L1210 leukemia cell lines *in vitro* is necessary to continue to test *in vivo* in mice inoculated with tumor, prior to clinical trials for the potential development of alkaloid compounds $C_{39}H_{64}NO_{11}$ as a natural anti-cancer drugs from local plants .

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