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 C39H64NO11 alkaloid isolated from the ethanol extract of fruit ranti hitam potentially bioactive. This study aims to determine the potential anticancer alkaloid C39H64NO11 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 C39H64NO11 alkaloid of the ethanol extract of fruit ranti hitam potentially bioactive anticancer against leukemia cells in L1210 with a value inhibition concentration 50 (IC50) of 1.2738 µg/mL < 4.0 µg/mL. As a positive control used Doxorubicin with IC50 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**
**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 $\text{C}_{39}\text{H}_{64}\text{NO}_{11}$ 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 nitrogen (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 improved, 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 NaHCO3 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 $2 \times 10^5$ cells/mL. L1210 leukemia cells used in this study were obtained from The Institute of Physical and Chemical Research of Japan (RIKEN).

**Inhibitory Power Test.** Test of inhibition of L1210 leukemia cell growth of alkaloid compounds $\text{C}_{39}\text{H}_{64}\text{NO}_{11}$ fruit ranti hitam done with dose variation of 0.5; 1.0; 2.0; 4.0 and 8.0 $\mu$g/mL of methanol. As a positive control used Doxorubicin dose variation of 0.08; 0.16 and 0.31 $\mu$g/mL. As a negative control is methanol (solvent sample). Media who had conceived L1210 leukemia cell suspension ($2 \times 10^5$ cells/mL) and samples $\text{C}_{39}\text{H}_{64}\text{NO}_{11}$ 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 10 $\mu$L of methanol was added 990 $\mu$L 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 % CO2 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. IC50 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 IC50 value ≤ 4 μg/mL.

RESULTS AND DISCUSSION

The test results from the inhibition of alkaloid compounds C39H64NO11 ranti hitam fruit and Doxorubicin as a positive control against L1210 leukemia cell growth are presented in Table 1.

<table>
<thead>
<tr>
<th>No.</th>
<th>Test material</th>
<th>Dose (µg/mL)</th>
<th>Inhibition (%)</th>
<th>IC50 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids C39H64NO11 (fruit ranti hitam)</td>
<td>0,50</td>
<td>36,21</td>
<td>1,2738</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1,0</td>
<td>46,5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2,0</td>
<td>56,90</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4,0</td>
<td>67,24</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8,0</td>
<td>74,14</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Doxorubicin (positive control)</td>
<td>0,08</td>
<td>37,93</td>
<td>0,1540</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0,16</td>
<td>51,72</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0,32</td>
<td>62,07</td>
<td></td>
</tr>
</tbody>
</table>

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).
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).

\[
\text{Log Concentration (x)} \quad \text{Probit (y)}
\]

\[
y = 0.8371x + 4.912
\]

\[ R^2 = 0.9967 \]

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

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

<table>
<thead>
<tr>
<th>No.</th>
<th>Test material</th>
<th>Conversion value</th>
<th>( \mu g/mL )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids compounds ( C_{39}H_{64}NO_{11} ) (fruit ranti hitam)</td>
<td>( r )</td>
<td>0.9980</td>
</tr>
<tr>
<td></td>
<td>( \text{Log } IC_{50} )</td>
<td></td>
<td>0.1051</td>
</tr>
<tr>
<td></td>
<td>( IC_{50} )</td>
<td></td>
<td>1.2738</td>
</tr>
<tr>
<td>2.</td>
<td>Doxorubicin (positive control)</td>
<td>( r )</td>
<td>0.9955</td>
</tr>
<tr>
<td></td>
<td>( \text{Log } IC_{50} )</td>
<td></td>
<td>-0.8124</td>
</tr>
<tr>
<td></td>
<td>( IC_{50} )</td>
<td></td>
<td>0.1540</td>
</tr>
</tbody>
</table>

Alkaloid compound \( C_{39}H_{64}NO_{11} \) \( IC_{50} \) of 1.2738 (\( \mu g / mL \)) showed concentrations of alkaloid compounds \( C_{39}H_{64}NO_{11} \) 1.2738 (\( \mu g / mL \)) 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} alkaloïd isolated from the ethanol extract of fruit ranti hitam potentially bioactive anticancer against leukemia cells in 1210 with a value inhibitory 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} alkaloïd 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 alkaloïd compounds C_{39}H_{64}NO_{11} as a natural anti-cancer drugs from local plants.

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REFERENCES


