IDENTIFICATION AND CYTOTOXIC ACTIVITY FROM N-HEXANE FRACTION OF BRUGUIERA GYMNORHIZA TO HELA CELLS AGAINST

INTRODUCTION
The discovery of new drug compounds from natural materials further clarify the crucial role of plant the secondary metabolites as source of raw material for medicine. Secondary metabolite is the compound result of biogenesis of primary metabolites. Commonly produced by the higher plants, which is not a determinant of the survival of compound directly, but rather as the result defense mechanism of organisms.

The mangrove plants are a source of bioactive compounds that can be used as source material of chemotherapy drugs such as tannins, saponins, terpenoids, alkaloids and steroids with activity anti-microbial, antifungal, antiviral, anticancer, insecticide and antileukemia (Soetarno, 2000). Ethanol extract of stem bark B. gymnorrhiza have cytotoxic activity to Raji cells with LC50 values of 301.78 µg/ml and Myeloma cells with LC50 of 582 µg/ml in vitro (Warsinah et al., 2005), the ethanol extract of the bark and leaves of R. mucronata can inhibit the growth of cancer cells, respectively Myeloma with LC50 of 91.49 µg/ml and 28.72 µg/ml (Diastuti et al., 2008), the flower B. gymnorrhiza contain compounds isobrugueriol, Bruguisulfurol and can inhibit the enzyme Cox -2 (IC50 6.1ug/ml (Homhual et al., 2006). methanol extract has cytotoxic activity to hela cells with IC50 288.78 µg/ml (Warsinah et al., 2008), the flower B. gymnorrhiza contain compounds isobrugueriol, Bruguisulfurol and can inhibit the enzyme Cox -2 (IC50 6.1ug/ml (Homhual et al., 2006). methanol extract has cytotoxic activity to hela cells with IC50 288.78 µg/ml (Warsinah et al., 2008), the purpose of this study is to identify the fraction of n-hexane and determine the cytotoxic activity to HeLa cells cultured in vitro.

RESEARCH METHODS

a. Materials Research
All material used in this study to pro quality analysis (merck), unless otherwise stated, the water used is distilled water. The main ingredient B gymnorrhiza bark taken from Cilacap on 18 May 2008. Solvents for the fractionation of n-hexane.Materials for cytotoxic assay consisting of HeLa cell cultures (from the Faculty of Medical Parasitic laboratory UGM), RPMI media (RPMI 1640 (Sigma), sodium bicarbonate (Ebewe / ebedoxo stock 10mg/ml)

b. Procedures
1. Fractionation of methanol extract Fractionation of 100 grams material of methanol extract with n-hexane solvent for 4 hours, stirring 3x 24 hrs perfectly soluble compounds, vaporized fraction obtained by evaporating the solvent evaporator until all. Fractions obtained weighed.

2. Cytotoxic Test
a. Preparation of test solution
A total the fraction of 10 mg was dissolved in 100 mL DMSO stock solution thus obtained 10 000 µg/mL. Then made series fractions levels of stock solution in RPMI 1640 media with graded dilutions to obtain concentrations of 500; 250; 125; 62.5 and 31.25 µg/ml.

b. Sitotoksis activity assay with MTT method
A total 100 ul cell suspension with a density of 2 x 104/100 ul distributed into the wells at 96 - well plate, concentration sample at each level 500; 250; 125; 62.5 and 31.25 µg/ml. As a control used 100 1 cell suspension in media. Further incubation for 24 hours. At the end of incubation into the wells added 1 5 MT MTT 5 mg/ml in RPMI. Then incubated 4 hours at 37 ° C. The reaction of MTT was stopped by the reagent stopper. Incubation the overnight at room temperature, absorbance is reading used elisa reader at a wavelength of 595 nm.

c. Apoptosis induction assay (Double staining)
Cells with 5x104 cells (200 l) distributed in wellplate 24 completed coverslip, RPMI medium was then added (800 l). Incubation in CO2 incubator, 37 ° C for 24 hours. Then the media removed and replaced with the next incubasi 24 hours. At the end of the incubation medium were taken, the cells were fixed with formaldehyde and added in 0.1% acridine orange. Tues fluorosensi observed under a microscope.

3. Identification of compounds in n-hexane fraction
Identification of the GC – MS
Samples were introduced into the mass spectrometer system revenue footage, in spectra observed mass is formed and its molecular weight fragments with reference spectra.

4. Analysis of data
1. Cytotoxic activities
Data obtained from the cytotoxicity assay was calculated using the formula:
\[ \% \text{Livining Cells} = \frac{\text{Ab}(P-M) - \text{Ab}(K-M)}{\text{Ab}(K-M)} \times 100\% \]
Notation:
P: absorbance media
K: absorbance of control cells
M: absorbance media

Then proceed with the analysis of the correlation test using the probit regression line equation and determine the IC50 values.

2. Induction of apoptosis
The data obtained were analyzed with qualitative diskritif

3. Identification of n-hexane fraction
Data GC-MS was interpreted and matched with the literature.

RESULT AND DISCUSSION
1. Fractionated extracts
Methanol extracts were fractionated by solid partitions method - uses a

ABSTRACT
Bruguiera gymnorrhiza is one plant of mangroves that has not been studied as the anticancer potential. Previous research the methanolic extract of stem bark B. gymnorrhiza is cytotoxic to HeLa of cancer cells against. The purpose of this research was done by the method fractionation methanol extract of liquid- solid partition with n-hexane solvent. Frations were tested cytotoxic activity to HeLa cells by MTT method. The results showed that the fraction of n-hexane have cytotoxic activity with IC50 of 253.3 µg / ml and the identification showed the fatty acids and terpenoids which can be lethal cells by apoptosis.

KEYWORDS
Bruguiera gymnorrhiza, fractionation, cytotoxic, HeLa cells

Pharmacy
Warsinah* Department of Pharmacy, Faculty of Health sciences University of Jenderal Soedirman Purwokerto Central Java, Indonesia *Corresponding Author

Hartiwi Diastuti Departemen of Kimia, Faculty of MIPA, University of Jenderal Soedirman Purwokerto, Central Java, Indonesia

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Based on the microscopic observation of cell morphology showed a difference between control and treated cells. Cell control (Fig. 1B) attached to the bottom of wells and shaped like a leaf while cells by treatment with n-hexane fraction showed morphological changes in the concentration of 250 µg/ml (Fig. 1C), treatment of cells with these fractions are dead, deformed, floats and murky. The cell viability data for the calculation of further diguankan LC₅₀ with Probit analysis method.

Based on the results obtained cytotoxic activity HeLa cells in vitro produces 17 peak (Figure 5), peaks were detected in the GC, not all is able to inhibit the growth of tumor cell lung (A-549) and colon cancer (HCT116) compounds. According to Cassady et al., (1990). Diterpene dilakton regulators of apoptosis (P53) bound and degraded by the E6 protein of HPV (Desaintes et al., 1999). Warsinah (2005), reported that in the methanol extract of the bark of B. gymnorrhiza containing terpenoid compounds. According to Cassady et al., (1990). Diterpene dilakton able to inhibit the growth of tumor cell lung (A-549) and colon cancer cells in vitro.

3. Identification of compounds N-hexane fraction

The results of the identification of compounds with GC content produces 17 peak (Figure 5), peaks were detected in the GC, not all is cytotoxic. Only 5 compounds with potential anticancer (Table 1)

CONCLUSION

Based on the results obtained cytotoxic activity HeLa cells in vitro with IC₅₀ 253 µg/ml, groups fraction contains fatty acids and terpenoids which can cause cell death by apoptosis.

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graphs the effects of treatment fractions on HeLa cells at levels of 500, 250, 125, 62.5, and 31.25 µg/ml cell morphology without treatment (A), n-hexane fraction treatment levels of 500 µg/ml (B), 250 µg/ml (C), 125 µg/ml (D) and 62.5 µg/ml (E), normal cells and cells treated.

3. Induction of apoptosis

Apoptosis induction assay performed with cell morphology observation by using ethidium bromide staining DNA with a concentration fraction based on price IC₅₀nya. The results can be observed under a microscope painting fluoresensi (Chow and Bogdan, 1977). The test results of induction apoptosis fraction n - hexane showed that the fraction has the ability to induce apoptosis in HeLa cells. Cells undergoing apoptosis and presumed dead be colored orange and green living cells (Figure 3). Apoptosis can be observed in the appearance of the physiological form of cell shrinkage, plasma membrane breakdown and chromatin condensation (Choi, 2004 and Berninghausan, 1997)

REFERENCE

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12. Soetarno, S.2000. Potential and Benefits of Mangrove Plants as Sources Bioactive

Figure 1. Fraction treatment effect on the growth of HeLa cells (A)

Tabel 1. compound potensial of anticancer with Gas Chromatograpfi

<table>
<thead>
<tr>
<th>No</th>
<th>Peak Name compounds</th>
<th>R. Time</th>
<th>% Area</th>
<th>Mass Peak</th>
<th>Rumus Molekul</th>
<th>Group compound</th>
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<tr>
<td>1</td>
<td>hexadecanoic acid, metil ester (metil palmiati)</td>
<td>14.675</td>
<td>13.05</td>
<td>89</td>
<td>C17H32O2</td>
<td>Fatty acid</td>
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<td>Fatty acid</td>
</tr>
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<td>3</td>
<td>9-octadecenoic acid, metil ester (metil elaidat)</td>
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<td>16.803</td>
<td>51</td>
<td>C19H36O2</td>
<td>Fatty acid</td>
</tr>
<tr>
<td>4</td>
<td>Metil isostearat</td>
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<td>12.04</td>
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<td>18.692</td>
<td>30.31</td>
<td>88</td>
<td>C15H24</td>
<td>Terpenoid: Sesquiterpen</td>
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Figure 2. HeLa cells are thought to undergo apoptosis (white arrow) and the living (yellow arrows) staining results of acridine orange and ethidium bromide control (A), cells treated with doxorubicin (B), the fraction of cells with n-hexane C. Overall the test results of induction of apoptosis by DNA staining method can support antiproliferative test conducted previously. Green control cells and apoptosis does not occur, it is because of the regulators of apoptosis (P53) bound and degraded by the E6 protein of HPV (Desaintes et al., 1999). Warsinah (2005), reported that in the methanol extract of the bark of B. gymnorrhiza containing terpenoid compounds. According to Cassady et al., (1990). Diterpene dilakton able to inhibit the growth of tumor cell lung (A-549) and colon cancer cells in vitro.

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