



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

Pharmacy

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ABSTRACT

Bruguiera gymnorhiza is one plant of mangroves that has not been studied as the anticancer potential. Previous research the methanolic extract of stem bark *B. gymnorhiza* 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. Fractions were tested cytotoxic activity to HeLa cells by MTT method. The results showed that the fraction of *n*-hexane have cytotoxic activity with IC_{50} of 253.3 μ g / ml and the identification showed the fatty acids and terpenoids which can be lethal cells by apoptosis.

KEYWORDS

Bruguiera gymnorhiza, fractionation, cytotoxic, HeLa cells

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 antimicrobial, antifungal, antiviral, anticancer, insecticide and antileukemia (Soetarno, 2000). Ethanol extract of stem bark *B. gymnorhiza* have cytotoxic activity to Raji cells with LC50 values of 301.78 μ g/ml and Myeloma cells with LC50 of 582 μ g/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 μ g/ ml and 28.72 μ g/ml (Diastuti, *et al.*, 2008), the flower *B. gymnorhiza* contained compounds isobruguerol, Bruguierosulfuro and can inhibit the enzyme Cox -2 (IC_{50} 6.1 μ g/ml (Homhual *et al.*, 2006). methanol extract has cytotoxic activity to hela cells with IC_{50} 288.78 μ g / 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. gymnorhiza* 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 (Sigma) and hepes (Sigma)), fetal bovine serum (FBS) (Gibco) 10% (v/v), penicillin-streptomycin (Gibco) 1% (v/v), fungison (Gibco) 0.5% (v/v), sterile distilled water, DMSO, acridine orange, ethidium bromide, sodium dodecyl sulfate (SDS), stopper reagent (sodium dodecyl sulfate) (merck) 10% in HCl), MTT (sigma), Doxorubicin HCl (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 μ g/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 μ g/ml.

b. Sitotoksis activity assay with MTT method

A total 100 μ l cell suspension with a density of 2×10^4 /100 μ l distributed into the wells at 96 - well plate, concentration sample at each level 500; 250; 125; 62.5 and 31.25 μ g/ml. As a control used 100 μ l cell suspension in media. Further incubation for 24 hours. At the end of incubation into the wells added 10 μ l 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 ellisa reader at a wavelength of 595 nm.

c. Apoptosis induction assay (Double staining)

Cells with 5×10^4 cells (200 μ l) distributed in wellplate 24 completed coverslip, RPMI medium was then added (800 μ l). Incubation in CO2 incubator, 37 ° C for 24 hours. Then the media removed and replaced with the next inubasi 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 flourosensi 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:

$$\% \text{Living Cells} = \frac{\text{Abs(P-M)} - \text{abs(K-M)}}{\text{Abs(K-M)}} \times 100\%$$

Notation:

P: absorbance Abuse

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 IC_{50} values.

2. Induction of apoptosis

The data obtained were analyzed with qualitative diskritifit

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

liquid solvent *n*-hexane. In early fractionation solvent yellow, fraction was stopped after clear colored solvent-soluble compounds *n*-hexane has extracted all of the total extract 100 grams obtained of *n*-hexane fraction each 4.85 grams. Further fractions tested activity HeLa cells against.

2. Cytotoxic test

The active fraction of the cytotoxic test. Cytotoxic effect was obtained by MTT assay that the incubating a variety of levels fractions. Levels of cytotoxicity the fraction to HeLa cells against was expressed also with IC₅₀ value. Based on the fraction of a percentage of the concentration curve of cell viability, demonstrating a correlation between the concentration of fractions with cytotoxic effects (Fig. 1A), indicating a linear relationship between the concentration of fractions with cytotoxic effects produced. In general, the data obtained showed the percentage of HeLa cell viability was compounded by the decline in the concentration of the test fraction (dose-dependent). Decrease in cell viability due to the provision of test fractions starting with different concentrations - different, This is because the test compound. contained in each of the different fractions thus providing different IC₅₀ values.

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 gave 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.

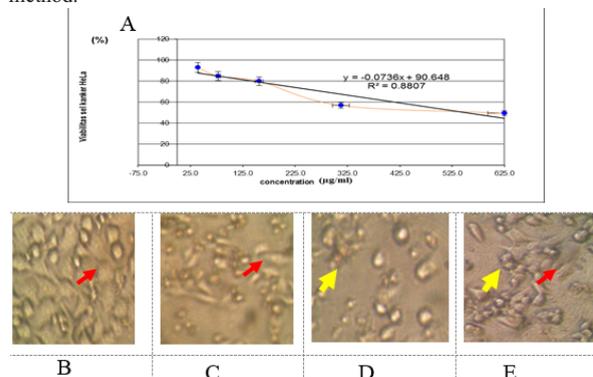


Figure 1. Fraction treatment effect on the growth of HeLa cells (A)

Table 1. compound potential of anticancer with Gas Chromatography

No	Peak	Name compounds	R. Time	% Area	Mass Peak	Rumus Molekul	Group compound
1	4	hexadecanoic acid, metil ester (metil palmitat)	14,675	13,05	89	C17H32O2	Fatty acid
2	5	Hexadecanoic acid	14,883	18,97	47	C17H34O2	Fatty acid
3	10	9-octadecenoic acid, metil ester (metil elaidat)	16,803	16,803	51	C19H36O2	Fatty acid
4	11	Metil isostearat	17,042	12,04	49	C19H38O2	Fatty acid
5	15	Cyclo hexan	18,692	30,31	88	C15H24	Terpenoid: Sesquiterpen

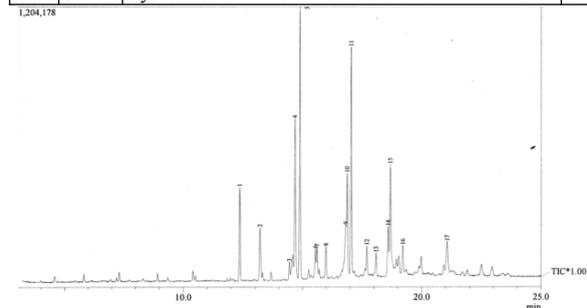


Figure 3. Spectrum Gas Chromatography

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.

ACKNOWLEDGMENTS

Author thanks goes to the Director General of Higher Education that has funded this research through doctoral research grant scheme

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 µ/ml (B), 250 µg/ml (C), 125 µg/ml (D) and 61.25 µ/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 are 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 Berninghausen, 1997)

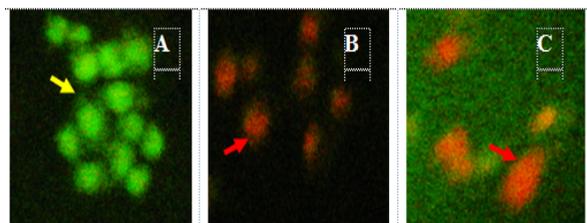


Figure 2. HeLa cells are thought to undergo apoptosis (white arrow) and the living (yellow arrows) staining results of acridine orange and ethidium bromide control (A), cells treated with doxorubicin (B), the fraction of cells with *n*-hexane (C).

Overall the test results of induction of apoptosis by DNA staining method can support antiproliferative test results 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. gymnorhiza* containing terpenoid compounds. According to Cassidy et al, (1990). Diterpene dilakton able to inhibit the growth of tumor cell lung (A-549) and colon cancer cells in vitro.

4. 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)

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