Identification and Citotoxic of....

by Warsinah -

ORIGINAL RESEARCH PAPER

Volume-8 | Issue-4 | April-2019 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

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

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

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 ug / 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 anti-microbial, antifungal, antiviral, anticancer, insecticide and antileukemia (Soetarno, 2000). Ethanol extract of stem bark *B. gymnorhiza* havecytotoxic activity to Raji cells with LC50 values of 301.78 µg/ml and Myeloma cells with LC50 of 582 ug/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 ug/ ml and 28.72 ug/ml (Diastuti, et al., 2008), the flower *B. gymnorhiza* contained compounds isobruguierol, 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 microg / 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 µg/ml. Then made series fraction 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 l cell suspension in media. Further incubation for 24 hours. At the end of incubation into the wells added 10 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 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 inbubasi 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 activitys

Data obtained from the cytotoxicity assay was calculated using the formula:

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

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 IC50 values.

2. Induction of apoptosis

The data obtained were analyzed with qualitative diskriftif

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 fractination 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 ug/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 LC_{50} with Probit analysis method.

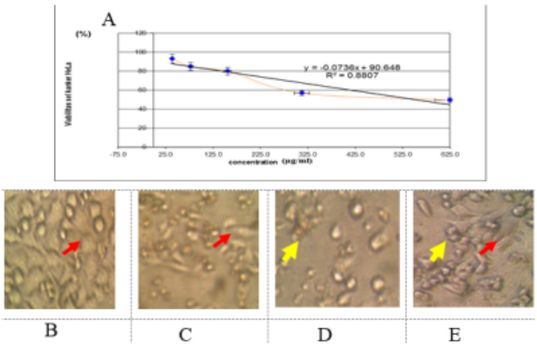

Figure 1. Fraction treatment effect on the growth of HeLa cells (A)

Tabel 1. compound potensial of anticancer with Gas Chromatografi

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

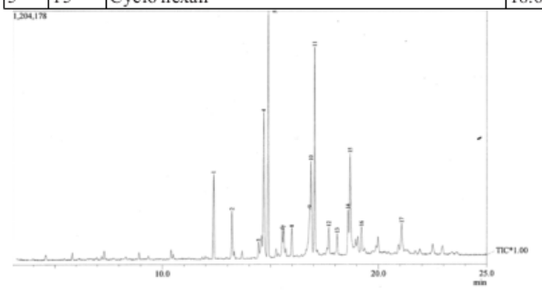

Figure 3. Spectrum Gas Chromatography

CONCLUSION

Based on the results obtained cytotoxic activity HeLa cells in vitro with IC50 253µg /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 500µ /ml (B), 250 ug/ml (C), 125 ug / 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 flouresensi (Chow and Bogdan, 1977). The test results of induction apoposis 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)

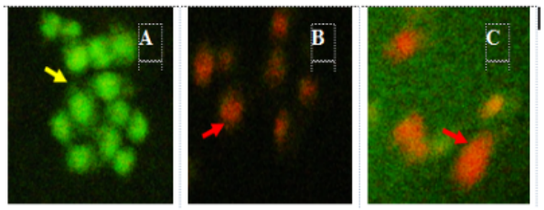

Figure 2. HeLa cells are thought to undergo apoptosis (white arrow) and the living (yellow arrows) staining results of acridine orange and ethidium bromide control (A), cells treated with doxorubicin (B), the fraction of cells with n-hexane ©,

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 Cassady 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)

REFERENCES

1. Anonim, 1983, American Type Culture Collection, 61.107, rockville, Maryland
2. ------. 2005a. Health Information for World. World Health Organisation. (Online). http: //diahome.org/content/abstract/2005/dij991.pdf. diakses 28 April 2018.
3. Cardenas ME, Sanfridson A, Cuter NS, and Heitman J, 1998, Signal Tranduction Cascade as targets For Therapetics Intervevtion by natural Products. Tibtech. Vol 16. October, 427 - 433
4. Cassady,, J.M., Baird, W., and Chang, CJ, (1990), Natural products as a Source of Potential Cancer Chemotherapeutic and chemopreventive Agents, Journal of Natural products, 53(1) 34.
5. Berninghausan,O, and Lippe, M. 1997. Necrosis versus apoptosis as the mechanism of target cell death induced by entamoeba hystolytica, infection and immunity. 65(9): 3615-3620
6. Chen. G., Zhu. Y., Wang. H.Z., Wang. S.J., Zhang. R. Q., The Metabolites of a mangrove endopathytic fungus, penicillium thomi, J. Asian Natural Product Research, 9: 159-164,
7. Choi, H.J., Bae, S.J., Kim, N.D., Jug, J.H and choi, Y.H, 2004, Induction of apoptosis by dideoxypetrosynol A. a polyasetylene from the sponge petrosia sp in human skin melanoma cells, int J. Mol. Med 14(6):1091-1096
8. Desaintes, C., Goyat, S., Garbay, S., Yaniv, M. and Thiery, F., 1999, Papillomavirus E2 Induces p53-independent Apotopsis in Hela Cells, Oncogene 18, 4583-4545
9. Homhual. S., Zhang. H.j., Bunyapraphatsara. N., Kondratyuk. T.P., SantarSiero B. D., Mesear A. D., Herunsalee A. Chaukul W., Pezzuto J.M., Fong H.H., 2006, Brugiesulfurol, a new compound from Bruguiera gymnorrhiza, Planta med, Departement of Pharmacognosy, Fakulty of Pharmacy Mahidol University, Bangkok, Thailand.
10. Ma'at, S. 2004. Traditional Medicines for Formal Health Care, Proceedings of the National Seminar, Tanggal 5 September 2004 di Surabaya pp. 45-49.
11. Sladowsky, D., Steer , S., J., Clothier, R., H., and Balls., M, 1992, An Improved MTT Assays Immunological Methods, p.157, 203-207
12. Soetarno, S.2000. Potential and Benefits of Mangrove Plants as Sources Bioactive

Materials.Acta Pharmaceutica Indonesia. 12 (4) : 84-103.
13. Warsinah, Puji Lestari, Trisnowati, 2005, Isolation of Bioactive Compounds in Leather Stem B. gymnorhiza As Anticancer Materials. Basic Research Reports.
14. Wyllie, A., Donahue, V., Fisher, B., Hill, D., Keesey, J. and Manzow, S., 2000, Cell Death Apoptosis and Necrosis. Rosche diagnosis Coorporation, 2-4

.

Identification and Citotoxic of....

ORIGINALITY REPORT

10%	10%	2%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	garuda.kemdikbud.go.id Internet Source	3%
2	worldwidejournals.co.in Internet Source	2%
3	pinpdf.com Internet Source	1%
4	phcogj.com Internet Source	1%
5	www.bioscmed.com Internet Source	1%
6	link.springer.com Internet Source	1%
7	www.ispeco.org Internet Source	1%
8	docsdrive.com Internet Source	1%
9	vdocuments.site Internet Source	1%

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		