Indonesian Journal of Cancer Chemoprevention, February 2011
ISSN: 2088–0197
e-ISSN: 2355-8989

Cytotoxicity Assay From Fractions of *Hedyotis corymbosa* Extract Against Breast Cancer Cell Line T47D

Rina Andriyani[1*], Chandra Risdian[1], Zalinar Udin[1]

[1]Research Center for Chemistry, Indonesian Institute of Sciences,
Jl. Cisitu-Sangkuriang, Gedung 50, Bandung-Indonesia 40135

Abstract

Drug discovery for cancer medication is the most important effort that researcher do at this time. Indonesia bio diversities have possibility as a cancer medicine sources. Finding a herbal medicine for cancer treatment is a first step to find a right cancer medicine in the future. This research has already completed for the earlier another research. Some fractions of *Hedyotis corymbosa* extract has been analyzed using Sulforhodamine B method with UV wavelength 515 nm against T47D cell line, a human breast cancer. There are Hexane extract, Methylene chloride extract and Ethyl acetate extract, and give inhibitory concentration 50 (IC_{50}) of 33.45 µg/ml, 54.59 µg/ml and 52.58 µg/ml, respectively. Ethanolic extract, itself has IC_{50} of 61.57 µg/ml, whereas IC_{50} value of Cisplatinum is 9.63 µg/ml. There is a difference between the ethanolic extracts with the other fraction.

Keywords: breast cancer, herbal medicine, T47D, Hedyotis corymbosa

INTRODUCTION

Drug discovery for cancer medication is the most important effort that researcher do at this time. Indonesian bio diversities have possibility as a cancer medicine sources. Finding a herbal medicine for cancer treatment is a first step to find a right cancer medicine in the future.

Hedyotis corymbosa is one of species from *Hedyotis* (genus), *Rubiaceae* (family), *Rubiales* (ordo), *Dicotyledoneae* (class), *Angiospermae* (sub-division), and *Spermatophyta* (Division). They grow well on dry and sandy soil, along rivers and coasts and in the forests (Ahmad, 2004). Beside they widely grow in Indonesia, they will widely found also in Malaysia and India. Rohaya Ahmad, 2004, also said that previous studies on some *Hedyotis* species have yielded indole alkaloids, anthraquinones, lignans, triterpenes, flavonoids as well as iridoids. In addition, three new iridoid glycosides are hedycorysides A-C has already found too (Wei Jiang, 2007).

Investigation to methanol extract of *Hedyotis corymbosa* resulted that extract have some bioactivities. Antibacterial activities, anti-inflammatory, their radical scavenging, cytotoxic and hepatoprotective had studied (Ahmad *et al*., 2004; Sadasivan *et al*., 2006). This investigation, we aim to investigate cytotoxity ethanol extract and its fractions of *Hedyotis corymbosa*. We use Sulforhodamine B method against T47D cell line to know their Inhibition Concentration 50 (IC_{50}).

METHODS

Plant materials and preparation of extract

The whole plants of *Hedyotis corymbosa* were collected from Indonesian Medicinal and Aromatic Crops Research Institute. It determined by Research Center for Biology, Indonesian Institute of Sciences.

*Corresponding author email: titiksunarni@yahoo.co.id

The whole plants were washed, dried and powdered. Maceration is done during 72 hours to gain crude extract, using ethanol technical quality as a solvent. More over to gain some fractions, from crude extract simultaneously extraction liquid-to-liquid using hexane, methylene chloride, and ethyl acetate.

Culture Cell

T47D (breast cancer) cell line was obtained from Pharmacy Department, Gadjah Mada University. Cells were cultivated in DMEM (GIBCO) medium supplemented with 10% v/v *Fetal Bovine Serum* (Sigma), 1% antibiotic-antimycotic (GIBCO). Cells maintained in $25cm^3$ flasks with 5 ml of DMEM at 37°C with 5% CO_2.

Assay for cytotoxic activity

Cytotoxic assay was determined using Sulforhodamine B method with UV wavelength 515 nm for measured. It is adapted from National Cancer Institute of America. Samples concentration ranged between 100–3.125 µg/ml. Each well filled with cell suspension in DMEM amount of 10^4 cells/ml. As a control positive is using Cisplatinum. The assay for each concentration of extract performed in triplicates and the culture plates incubate at 37 °C with 5% CO_2 for 24 hours. After 24 hours, old medium removed, washed with PBS solution, and added DMEM fresh medium. Samples with slightly concentration, added to the flasks, and then keep at 37°C with 5% CO_2. After 24 hours later, plates added cold 50% trichloroacetic acid, incubated at 4°C for 30 minutes, washed with tap water 5 times, and air dry plates until no standing moisture is visible. Next step, cell staining. Stain 100 µl/well of 0.4% SRB in 1% acetic acid for 30 minutes. Rinse off unbound dye with 1% acetic acid five times, air-dried. Solubilize bound dye with 200 µl/well 10 mM Tris base (pH10) for 5 minutes on a gyratory shaker. The measurement for Optical Density (OD) at 515 nm used ELISA plate reader.

RESULTS

Range concentration ethanol extract of *Hedyotis corymbosa* and its fractions showing percentage proliferation to T47D, between 3.125 - 100 µg/ml. The result is showing on Table 1 below. The result show that 12.5 µg/ml of Cisplatinum concentration as positive control equal to 100 µg/ml each concentration extracts of samples. Percent proliferation of ethyl acetate extract given the best value than the others on the same concentration, it is 28.79%.

Table 1. Percent Proliferation of Extract and Its Fractions of *Hedyotis corymbosa*

Samples	% Proliferation					
	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.125 µg/ml
Ethanolic extract	31.93	51.67	65.36	73.63	92.16	82.04
Hexane extract	34.43	40.27	43.69	71.06	91.87	94.16
MTC extract	34.93	49.32	73.06	88.09	75.98	81.39
Ethyl acetate extract	28.79	50.53	76.76	93.65	46.69	62.22
Cisplatinum	42.68	35.63	34.69	32.10	74.63	82.93

Linear regression used to analyze about correlation between concentration samples and proliferation percentage T47D cell line. Base on linear regression analyzing, we can know a value of Inhibition Concentration 50 (IC_{50}) from each samples. Some graphics are shown below, from Figure 1 to figure 5. Hexane extract Of *Hedyotis corymbosa* given the best IC_{50} value, it is 33.45 µg/ml. All IC_{50} data are able to see in Table 2.

Table 2. Inhibition Concentration 50 (IC_{50}) of Extract and Its Fractions of *Hedyotis corymbosa* to T47D Cells

Sample	Inhibition Concentration 50 (IC_{50}) µg/ml
Ethanol extract	61.57
Hexan extract	33.45
MTC extract	54.59
Ethylacetat extract	52.58
Cisplatinum	9.63

DISCUSSION

The present study reports the cytotoxicity of *Hedyotis corymbosa* extract and its fractions against T47D breast cell line. Bioassay for the crude extract of *Hedyotis corymbosa* has been reported have antioxidant, radical-scavenging, anti-inflammatory, cytotoxic, antibacterial activities and hepatoprotective (Ahmad *et al*., 2004 and Sadasivan *et al*., 2006). They reported that methanol extract of *Hedyotis corymbosa* has cytotoxicity against CEMS-SS with CD_{50} value 21 µg/ml. CEMS-SS is a human T-lymphoblastoid cell line. Our investigation resulted that ethanol extract of *Hedyotis corymbosa* has cytotoxicity against T47D cell line, a human breast cancer, with IC_{50} value 61 µg/ml. IC_{50} value for its fraction shown a different activity. The value is better than the crude extract. It could be compounds in the crude extract have antagonism and compounds are able to synergism in fractions.

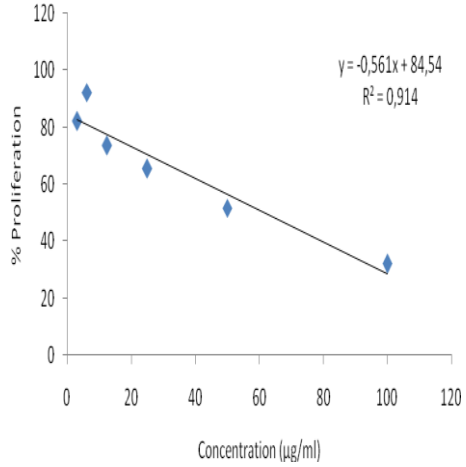

Figure 1. Linear Regression of Ethanol Extract

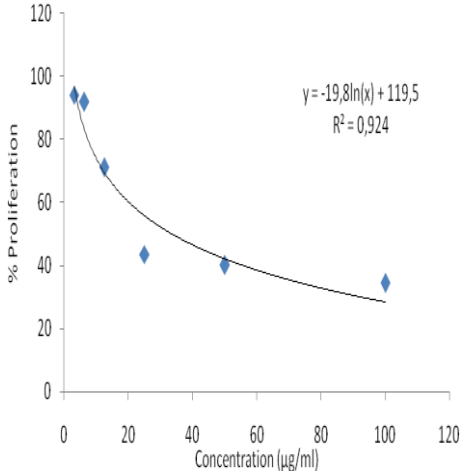

Figure 2. Linear Regression of Hexane Extract

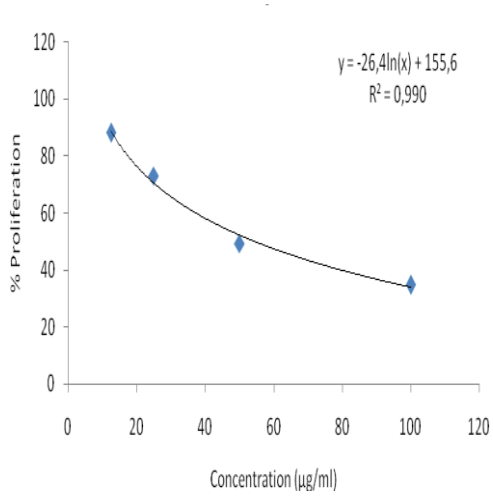

Figure 3. Linear Regression of MTC Extract

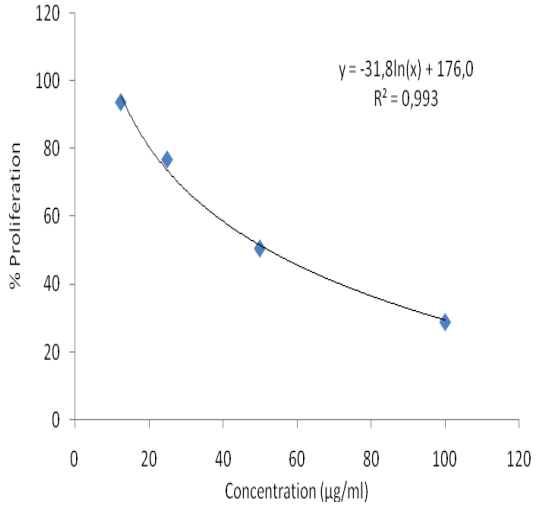

Figure 4. Linear Regression of Ethylacetate Extract

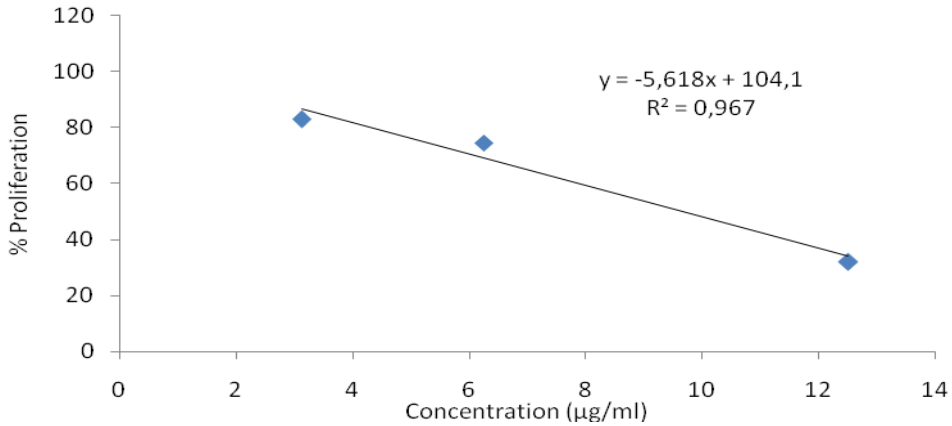

Figure 5. Linear Regression of Cisplatinum

The former studied by the other researcher, *Hedyotis corymbosa* contains some compounds. There are oleanolic acid, ursolic acid, sitosterol, stigmasterol, asperglaucide, indole alkaloids, anthraquinones, lignans, triterpenes, iridoid glycosides are hedycorysides A-C (Liao *et al*., 1979; Tong-Ing Ho *et al*., 1986; Wei Jiang, 2007). There is possibility that activity, which is shown, affected from one of compounds. It should be study further to know specific active compound that causing active as anticancer for this herb.

CONCLUSION

We reported our studied to the crude extract (ethanol extract) of *Hedyotis corymbosa* and its fractions have Inhibition Concentration 50 (IC_{50}), 61.57 µg/ml and 33.45 µg/ml for Hexane fraction; 54.59 µg/ml for Methylene chloride; 52.58 µg/ml for Ethyl acetate.

ACKNOWLEDGMENT

The authors would like to thank the Director of Research Center of Chemistry for the fund provided under DIPA grant. Appreciation goes to Ms. Nur Hidayati for the great technical support at extraction process.

REFERENCES

Ahmad, R., Ali, A.M., Israf, D.A., Ismail, N.H., Saari, K. and Lajis, N.H., 2004, Antioxidant, radical scavenging, anti-inflamatory, cytotoxic and antibacterial activities of methanolic extracts of some *Hedyotis* species, *Life Sciences,* **76**(2005) 1953-1964.

Liou, W-C., Lin, Y-C., Y-M. and Chen, F-C. 1979, *Chemistry* (Taipei) 72.

Sadasivan, S., Latha, P.G., Sasikumar, J.M., Rajashekaran, S., Shyamal, S. and Shine, V.J., 2006, Hepatoprotective studies on *Hedyotis corymbosa* (L.) Lam., *J Ethnopharmacol.*, **106**(2), 245-249.

Tong-Ing, Ho., Chen, G.P., Lin, Y.C., Lin, Y.M. and Chen, F.C., An anthraquinone from *Hedyotis diffusa*, *Phytochemistry*, **25**(8), 1988-1989.

Wei Jiang, Li-Sha Kuang, Ai-Jun Hou, Min Qian, and Ji-Zong Li, 2007, Iridoid glycosides from *Hedyotis corymbosa, Helvetica Chimica Acta*, **90**(7), 1296-1301.