International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.6, No.6, pp 1806-1809, Oct-Nov 2014

Cytotoxic Effect of n-Hexane, Ethylacetate and Ethanol Extracts of *Plectranthus amboinicus*, (Lour.) Spreng.) on HeLa and Vero Cells Lines

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Abstract: *Plectranthus amboinicus* (Lour) Spreng. had potential anticancer activity. The aims of the study were to evaluated the cytotoxic activity of n-hexane, ethylacetate and ethanol extracts of *Plectranthus amboinicus* (Lour) Spreng. The cytotoxic assay was performed on HeLa and Vero cells lines, using MTT assay. The result were showed that the three extracts had cytotoxic effect on HeLa cell with IC_{50} values 76.322 µg/mL, 143.291 µg/mL, and 88.997 µg/mL, respectively . The n-hexane and ethanol extracts were found selective to HeLa cells.
Keywords: cytotoxic, *Plectranthus amboinicus* (Lour) Spreng., HeLa, Vero.

Introduction

Cancer is one of the major causes of death in many countries. Effective anticancer drug with selectivity againts only malignant cells and with ability to repress tumor metastasis are desired [1]. Research into plants with anticancer effects is still encouraged with a view to discover any new drugs with less toxic but more potent effects. *Plectranthus amboinicus* (Lour) Spreng., (*Coleus amboinicus*, Lour., *Coleus aromaticus*, Benth.), is one of plant that used as lactagogue by native people in north Sumatera, Indonesia. This plant has also been traditionally used for the treatment of inflammation [2], heart disease [3], diuretic [4], immunomodulator [5] and hepatoprotector [6]. Phytomedicines derived from plants have shown great promise in the treatment of cancer disease.

Recent studies have revealed that anticancer activity of *Plectranthus amboinicus* (Lour) Spreng. extracts on MCF7 and T47D may be related to the inhibition of cell cycle and induced cell death [7]. The aim of this study is to evaluate the cytotoxic activity of *Plectranthus amboinicus* (Lour) Spreng. leaves extracts on HeLa and Vero cells line.

Materials and Methods

Chemicals and reagents

n-hexane, ethylacetate and ethanol were purchased from Merck (Darmstadt, Germany), DMSO (Sigma Aldrich Chemie GmBH Germany), [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT) (Sigma Chemical, St. Louis, MO), RPMI media and Phosphate Buffer Saline (FBS) 10% v/v (Gibco, Grand Island, NY, USA).

Plant and preparation of Extracts

The *Plectranthus amboinicus* was obtained from Pematang Siantar city, North Sumatera province, Indonesia. The leaves of *Plectranthus amboinicus* were dried at 45^0C and ground into powder. The dried leaves powder (500 g) was extracted with n-hexane by maceration method. After three days of maceration at room temperature, the supernatant was separated by decantation and the marc was remacerated twice. the marc of n-hexane extract was extracted with etylacetate by maceration. The same procedure was applied to ethanolic extract. Extract from each solvent were concentrated by a rotary evaporator (Heidolph VV-200) and the concentrated extract was dried by freeze-dryer (Edwards).

Cell Lines and culture conditions

HeLa (cervical adenocarcinoma) and Vero (African green monkey kidney) cell lines were kindly provided by Parasitology Laboratory, Faculty of Medicine, Gadjah Mada University, Indonesia. The cell lines were cultured in RPMI medium, suplemented with 10% (v/v) foetal bovine serum (FBS), 2% penicillin-streptomycine and 0.5% fungizone in a 37^0C incubator with 5% CO_2.

Cytotoxic assay

Cytotoxic activity was determined by the MTT colorimetric assay. Briefly, HeLa and Vero cell lines were plated at 10^4 cells/well in a 96-well plate. The culture cells were incubated in a humidified incubator at 37^oC at atmosphere of 5% CO_2 and 95% air for 24 h. After incubation for 24h at 37^0C, the medium was discharged and cells were treated by 3 extracts *(*n-hexane extract, ethylacetate extract, ethanol extract of *Plectranthus amboinicus*) with different concentration and incubated for 24 h. MTT 0.5 mg/mL solution was added to each well and further incubated for 4 h at 37^0C. Viable cells react with MTT to produce purple formazan crystals. After 4 h, the stopper 10% SDS (Sigma Co. St. Louis) in 0.01 N HCl (Merck) was added to dissolve the formazan crystal. The cells were then incubated for 24 h in room temperature and protected from light. After incubation, the cells were shaken. Optical density was read with an ELISA reader at λ 595 nm.The experimental data was absorbance of each well, and then converted to percentage of viable cells.

$$\text{Percentage of viable cell} = \frac{B - C}{A - C} \times 100\%$$

Where A, B and C are absorbance of control group, treatment group and medium (vehicle), respectively.

Statistical analysis

All data were expressed as IC_{50} that analysed using probit in regression at SPSS 19, test were used for statistical analyses with *p* values 0.05 were considered significant.

Results

Cells were exposed to various concentration of *Plectranthus amboinicus* extracts (31.25 – 500 µg/mL) for 24 h. Cells treated with DMSO were used as control. In this MTT assay, decreased absorbance at 595 nm correlates with decreased viability of cells in culture. The higher concentration of extracts were showed the decreased of the cell viability. The extracts showed cytotoxic activities in a concentration dependent manner (Fig.1).

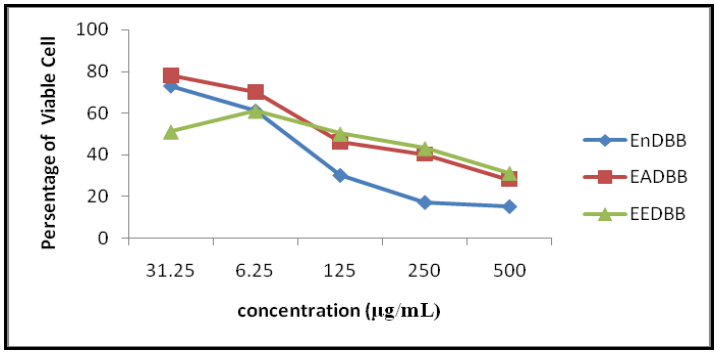

Figure 1. Cytotoxic effect of *Plectranthus amboinicus* extracts on HeLa cell line

The extracts were showed different result for Vero cell line. The ethanol extract of *Plectranthus amboinicus* (Lour.) Spreng. practically non toxic on Vero cell. High cell viability of ethanol extract was contrary to the ethylacetate extract (**Fig.2**).

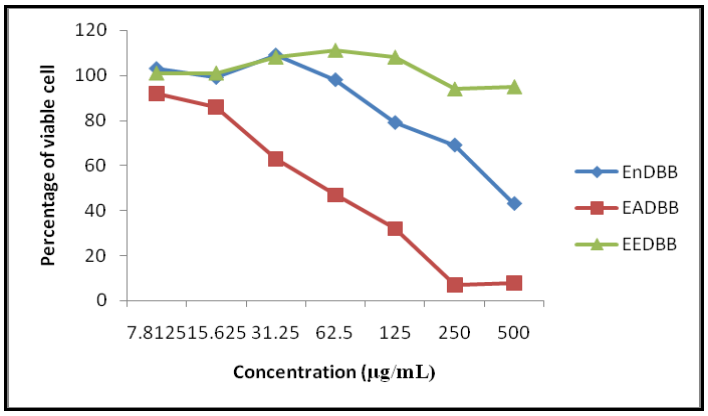

Figure 2. Cytotoxic effect of *Plectranthus amboinicus* extracts on Vero cell line

The cytotoxic activity potency were evaluated by IC_{50} value. The calculation of IC_{50} used probit analysis was found that the IC_{50} value of *n*-hexane, ethylacetate, and ethanol extract consecutively 76.322 µg/mL, 143.291 µg/mL, and 88.997 µg/mL. The decreased of IC_{50} value correlated with increased of cytotoxicity. The potential cytotoxic activity of the extract is less than 100 µg/mL [8]. While the *n*-hexane, ethylacetate and ethanol extracts of *Plectranthus amboinicus* had cytotoxic effect on HeLa cell, the effect of extract on Vero cell were showed different result. The *n*-hexane extract had slight cytotoxic on Vero with IC_{50} 414,467 µg/mL, but the ethylacetate was moderate cytotoxic on Vero cell with IC_{50} 55,972 µg/mL. Meanwhile, the IC_{50} of ethanol extract could'nt be calculated. the results were contrary to the previous study that strong inhibitory of ethylacetate extract was observed againts MCF7 and T47D cells lines with IC_{50} 7,647 µg/mL [7]. The reason for the activity difference may be attributed to the difference effect of soluble compound on each cell line.

To measure the selectivity of the extracts, we were executed cell viability assay on Vero cells. The IC_{50} of the extracts on Vero cell was compared to HeLa cell to find selectivity index (SI). The SI of n-hexane extract of *Plectranthus amboinicus* is 5,43. The SI > 3 is supposed to be selective to HeLa cell lines [9]. The ethylacetate extract of *Plectranthus amboinicus* was not selective because the result showed SI<3.

In conclusion, the extract of *Plectranthus amboinicus* were exhibited cytotoxic activity on HeLa cell line. The n-hexane and ethanol extracts had selectivity to HeLa cell line but the ethylacetate extract of *Plectranthus amboinicus* didn't.

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