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Research Article

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Combinational effects of n-hexane extract of poguntano leaves (*Picria fel-terrae* Lour.) with doxorubicin on MCF-7 breast cancer cells

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ABSTRACT

Poguntano (Picria fel-terrae Lour.) leaves were extracted using n-hexane and examined cytotoxicity on MCF-7 cell line. Analysis data using SPSS 19 was showed that n-hexane extract has IC_{50} 119,906 µg/ml and the value of cytotoxicity assay on Verro cell is < 3 showed that the extract is not selective. Cytotoxicity activity and combination of n-hexane extracted with doxorubicin were evaluated using the MTT assay. The combination represents higher inhibitory effect on cell growth than the single treatment of doxorubicin on MCF-7 cell lines.

Key words: Combination, *Picria fel-terrae* Lour., cytotoxicity, MCF-7, doxorubicin.

INTRODUCTION

The diversity of medicinal plants in Indonesia is one of chances in development potential of Indonesia in the globalization era [1]. The use of medicinal plants in the community is increasing in several decades [2,3]. Indonesia has thousands of islands with various plants in it and the manners of community using plants as treatment for every disease traditionally [1]. Poguntano (*Picria fel-terrae* Lour.) in east and southeast Asia has been used traditionally as a stimulant, diuretic, anti malaria, anti hyperglycemia, fever, herpes infection, cancer and inflammation for over 200 years [4]. Breast cancer is a type of cancer that most often affects women and leading cause of death in women, and based on the US data in 2010 breast cancer is the most common cancer with 209.060 new cases [5].

EXPERIMENTAL SECTION

Plant material

Fresh leaves of *Picria fel-terrae* Lour. were collected from Tiga Lingga village, Dairi regency, Sumatera Utara province, Indonesia. *Picria fel-terrae* Lour. was identified in Research Centre for Biology, Indonesian Institute of Science, Bogor and the voucher specimen was deposited in herbarium. Doxorubicin (Ebewe), DMSO (Sigma), [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT) (Sigma).

Preparation of n-hexane extract.

The air-dried and powdered leaves of *Picria fel-terrae* Lour. (1 kg) were repeatedly extracted by cold maceration with n-hexane (3x3 d, 7.5 L). The filtrate was collected, and then evaporated under reduced pressure to give a viscous extract and then freeze dried to give a dried extract [6].

Cytotoxicity assay

The combination of n-hexane extract and doxorubicin were submitted to cytotoxicity test. In that way, MCF-7 cell line was grown in DMEM medium, while Vero cell line was grown in M199 medium containing 10% Fetal Bovine Serum (Gibco), 1% penicillin-streptomycine (Gibco), and fungizone 0.5% (Gibco) in a flask in a humidified atmosphere (5% CO_2) at 37^{o}C. The inoculums seeded at 10^4 cells/mL at an optimal volume of 0.1 mL per well. After 24 h incubation, the medium was discharged and treated by extracts and doxorubicin. After incubation 24 h, the cells were incubated with 0.5 mg/mL MTT for 4 h in 37^{o}C. Viable cells react with MTT to produce purple formazan crystals. After 4 h, SDS 10% as stopper (Sigma) in 0.01N HCl (Merck) was added to dissolve the formazan crystals. The cells were incubated for 24 h in room temperature and protected from light. After incubation, the cells were shaken, and absorbance was measured using ELISA reader at λ 595 nm. The data which were absorbed from each well were converted to percentage of viable cells [8,9]. The selectivity index was calculated using equation where IC_{50} on Vero cells were divided with IC_{50} on MCF-7 cells [10].

Statistical analysis

All data were analyzed using regression using SPSS 19.

RESULTS AND DISCUSSION

This research was aimed to investigate the efficacy of n-hexane extract as a co-chemotherapy on doxorubicin treatment. N-hexane, doxorubicin and their combination were investigated for their cytotoxicity effect on MCF-7 cell lines, and selectivity was measured on Vero cells. MTT method was using to determined cell viability after incubation for 24 h. In every treatment (n-hexane, doxorubicin and their combination) was showed the inhibition of cells growth. The IC_{50} value of n-hexane 119,906 µg/mL and the combination was showed higher inhibitory effect if compare with single treatment. The optimum combination index (synergistic effect) was showed in 1/8 IC_{50} value of n-hexane extract and 1/8 IC_{50} value of doxorubicin (15 µg/mL - 50 nM) categorized with synergistic effect 0.55 (CI < 1).

To measure the selectivity of n-hexane extract, we were executed cell viability assay on Vero cells. Single treatment of n-hexane extract showed cytotoxicity effect on Vero cells with IC_{50} 175.644 µg/mL. We were compared IC_{50} of n-hexane extract on Vero cells to MCF-7 cells to find selectivity index (SI) [11]. SI of n-hexane extract is 1.5 (SI < 3), the result showed that n-hexane is not selective to MCF-7 cells instead of Vero cells.

The leaves of *Picria fel-terrae* Lour. are used in North Sumatera to treat hyperglycemia patient. Although some compound have been identified as possesing medicinal properties, none of these compounds has ever reached clinical trials. Moreover, the anticancer effect of *Picria fel-terrae* Lour. have not been validated in vitro to date based on their use in Indonesia or other system of medicine.

The cytotoxicity estimate of natural product is related to content of active compound in these plants including *Picria fel-terrae* Lour. Flavonoids and triterpenoids/steroids estimated as active compound[12]. We were evaluated the activity of n-hexane extract on cytotoxicity. We were also investigated selectivity of n-hexane extract on Vero cells and showed non selectivity on MCF-7 cells line if compared to Vero cells using SI value[13].

Doxorubicin is one of chemotherapeutic agent showing strong activity on MCF-7 cell lines with IC_{50} value of 375.804 nM. MCF-7 cells line undergo resistant to doxorubicin pass through to caspase-3 mutation [14],[15].

However, the molecular mechanism of apoptosis induction and cell cycle modulation by this extracts need to be explored more detail. Based on the results, we were concluded that n-hexane and ethylacetate extract of *Picria fel-terrae* Lour. leaves are potential to developed as co-chemotherapeutic agent in breast cancer therapy.

MTT method was using to determined cell viability after incubation for 24 h. In every treatment (n-hexane extract, doxorubicin and their combination) was showed the inhibition of cells growth. The IC_{50} value of n-hexane extract 119.906 µg/ml and the combination was showed higher inhibitory effect if compare with single treatment. The optimum combination index (synergistic effect) was showed in 1/8 IC_{50} value of n-hexane extract and 1/8 IC_{50} value of doxorubicin (15 µg/mL- 50 nM) categorized with synergistic effect 0.55 (CI < 1).

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