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Combinational effects of ethylacetate extract of *Zanthoxylumacanthopodium*DC. with doxorubicinon MCF7 breast cancer cells

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Abstract: Andaliman Fruit (*Zanthoxylum acanthopodium* DC.) Is one of the spices of wild plant known by the Bataknense, North Sumatera and is often used as a seasoning in various dishes. Andaliman fruit is widely used as a spice in cooking meat, and fish that become resistant dishes several dayswithout causing odor.
This research having purpose to evaluate the effects of ethylacetate extract (EAE) of *Zanthoxylum acanthopodium* DC. fruits and combination with doxorubicin on cytotoxicity on MCF-7cell lines.The in vitro cytotoxicity effects were determined using MTT assay. Cytotoxicity activity and combination of EAE 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.
Keywords: Andaliman fruit, *Zanthoxylum acanthopodium* DC, cytotoxic, ethylacetate, MCF-7.

1. Introduction

One typical Indonesian plant suspected to have potential as anticancer is the andaliman fruit (*Zanthoxylum acanthopodium* DC.). Andaliman fruit is a species of Zanthoxylum (Rutacea) are known locally andaliman (Toba) or sinyar-sinyar (Angkola). Andaliman fruit Traditionally used as seasoning in North Sumatra in particular North Tapanuli[1]. In previous research done on the plant genus Zanthoxylum generally prove that showed a cytotoxic effect against lung cancer cells, cervical cancer cells, colon cancer cells and breast cancer selective[2].

MCF-7 cells (Michigan Cancer Foundation-7) is a model of the cancer cells are often used. In MCF-7 cells, PGP expressed high, so the sensitivity of cells to chemotherapeutic agents such as doxorubicin low[3]. This concentration reduction can reduce the effectiveness of chemotherapy compounds in MCF-7 cells and to increase the sensitivity ofMCF-7 is by inhibiting the expression and activation of PGP[4]. There fore, it is necessary to use a combination therapy chemopreventive agent to increase the sensitivity of breast cancer cells MCF-7 against the chemotherapeutic agent doxorubicin.

2. Material and methods

2.1 Plant material

Fresh fruits of *Zanthoxylum acanthopodium* DC.were collected from Onan Rungu village, Samosir regency, Sumatera Utara province, Indonesia. *Zanthoxylum acanthopodium* DC. 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).

2.2 Preparation of ethylacetate extract (EAE)

The air-dried and powdered leaves of *Zanthoxylum acanthopodium* DC. (1 kg) were repeatedly extracted by cold maceration with n-hexane (3x3 d, 7.5 L). The powder were dried in the air and extracted with ethylacetate (3x3 d, 7.5 L) at room temperature on a shake. The filtrate was collected, and then evaporated under reduced pressure to give a viscous extract and then freeze dried to give a dried extract.

2.3 Cytotoxicity and combination assay

The combination of ethylacetate 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 EAE in combination with 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 [7]. The selectivity index was calculated using equation where IC_{50} on Vero cells were divided with IC_{50} on MCF-7 cells [8].

2.4 Statistical analysis

All data were analyzed using regression using SPSS 20.

3. Results

This research was aimed to investigate the efficacy of EAE as a co-chemotherapy on doxorubicin treatment. EAE, doxorubicin and their combination were investigated for their cytotoxicity effect on MCF-7 cell lines. MTT method was using to determined cell viability after incubation for 24 h. In every treatment (EAE, doxorubicin and their combination) was showed the inhibition of cells growth. The IC_{50} value of EAE 136.490 µg/ml and doxorubicin 348.72 nM, 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 EAE and 1/16 IC_{50} value of doxorubicin (17 µg/mL-25 nM) categorized with strong synergistic effect (CI <0.1).

4. Discussion

The fruits of *Zanthoxylum acanthopodium* DC. are used in North Sumatera to seasoning. Although some compound have been identified as possesing medicinal properties, none of these compounds has ever reached clinical trials. More over, the anti cancer effect of *Zanthoxylum acanthopodium* DC. 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 *Zanthoxylumacanthopodium* DC.Flavonoids and triterpenoids/steroids estimated as active compound[9]. We were evaluated the activity of ethylacetate extract on cytotoxicity and cell cycle of MCF-7 cells line with single treatment and in combination with doxorubicin[10].

Doxorubicin is one of chemotherapeutic agent showing strong activity on MCF-7cell lines with IC_{50} value of 348.72nM. MCF-7 cells line undergo resistant to doxorubicin pass through to p53 mutation [11],[12]. To decrease the toxic effect and prevent resistance from doxorubicin toMCF-7 cells, combination of small concentration of doxorubicin with EAE is required. In this study, combination of EAE and doxorubicin were showed very strong synergism activity on MCF-7cell lines. EAE was enhanced the cytotoxicity activity of doxorubicin on MCF-7 cell lines if compared to single treatment of either EAE or doxorubicin. The strongest synergistic effect was suggested to be related to a cell cycle modulation.

However, Based on the results, we were concluded that combination of ethylacetate extract of *Zanthoxylum acanthopodium* DC.fruits and doxorubicin very stong synergically increases the cytotoxicity activity of doxorubicin through cell cycle arrest. The extract is potential to developed as co-chemotherapeutic agent for doxorubicin in breast cancer therapy.

Conflict of interest statement

We declare that we have no conflict of interest.

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