



Ethylacetate Extract of *Zanthoxylum acanthopodium* DC. Fruit Against Doxorubicin-Resistant T47D Cells

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ABSTRACT

This study was carried out to investigate the activity of ethylacetate extract of *Zanthoxylum acanthopodium* DC., fruit (EEZ) against doxorubicin-resistant T47D cells. EEZ was prepared by maceration and T47D cells were treated by doxorubicin (DOX) for six times and IC_{50} was determined for each treatment. T47D cells were resistant with DOX with IC_{50} before 0.203 $\mu\text{g/mL}$ and 2.415 $\mu\text{g/mL}$ after 6th treatment. IC_{50} value of EEZ was determined with IC_{50} 48.760 $\mu\text{g/mL}$ before and 50.380 $\mu\text{g/mL}$ after 6th treatment. This results suggest that EEZ can use for chemoresistance cancer cell.

Keywords: Doxorubicin, resistance, *Zanthoxylum acanthopodium*, ethylacetate extract.

INTRODUCTION

The development of molecular targeted therapy in cancer is necessary to reduce the occurrence of cell resistance and toxicity towards normal cell. Therapeutic targets may involve many proteins and mechanisms, including the inhibition of protein in the signaling process which regulates the growth and the development of cancer cells and of proteins which causes the resistance of cancer treatment [1].

Traditionally, *Zanthoxylum acanthopodium* DC. fruits has been used as aromatic substances, tonic, and treat dysentery. Indian people have been used andaliman to treat paralyzed and skin disease such as abscess and leprosy. Andaliman have been used as spices at North Sumatera especially at North Tapanuli [2-4]. The plants from *Zanthoxylum* genus much contain compounds like phenol hydroquinones, flavonoids, steroids/triterpenoids, tannins, glycosides, volatile oils, alkaloids, coumarins, lignans, amides and terpenes [5-12]. Ethylacetate extract of andaliman fruits was showed cytotoxicity effect against MCF-7 and T47D cell lines. EEZ was presented synergistic effect when combined with doxorubicin. EEZ was showed anticancer activity towards mice which induction with benzo (a) pyrene and having cardioprotector effect [13,14].

Doxorubicin (DOX) is an anthracycline class of the most effective and broad-spectrum antineoplastic widely used as anticancer on various types of cancer including breast cancer [15,16] but the use of DOX is clinically irreversible cardiotoxic side effects and cause of death in cancer patients [17-20].

The purpose of this study was to investigate the activity of ethylacetate extract of *Zanthoxylum acanthopodium* DC., fruit (EEZ) against doxorubicin-resistant T47D cells.

MATERIALS AND METHODS

Materials

Doxorubicin (DOX) (Kalbe), *Zanthoxylum acanthopodium* DC fruit was obtained from Onan Rungu village, Samosir regency, Sumatera Utara province, Indonesia. n-hexane and ethylacetate were purchased from Merck (Darmstadt, Germany), DMSO (Sigma Aldrich, 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).

Preparation of EEZ

The air-dried and powdered fruit of *Zanthoxylum acanthopodium* DC. (1 kg) was repeatedly extracted by cold maceration with n-hexane (3x3 d, 7.5 L). The powder was 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[21-23].

Molecular evolution assay

Cells (80% confluency) were treated with drugs for 24 hours, as previously described [24]. Concentration of doxorubicin was 0.02 $\mu\text{g/mL}$ (1/10 of IC_{50} value). Medium was changed with fresh medium after drug exposure, and cells were grown until recovered (80% confluency again). As soon as recovered, cells then were splitted for cytotoxicity assay and for the next sequential treatment. R0 represents the untreated control cell line (parental cells), whereas R1, R2, R3, R4, R5 and R6 represent cells which are treated one, two, three, four, five and six times, respectively [24].

Cytotoxicity assay

EEZ and DOX were submitted to cytotoxicity test. In that way, T47D cell line was grown in RPMI 1640 medium containing 10% Fetal Bovine Serum (Gibco), 1% penicillin-streptomycin (Gibco), and fungizone 0.5% (Gibco) in a flask in a humidified atmosphere (5% CO_2) at 37°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 EEZ and doxorubicin. After incubation 24 h, the cells were incubated with 0.5 mg/mL MTT for 4 h in 37°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 [21,22,25,26,27].

Statistical analysis

All data were analyzed using regression using SPSS 22.

RESULTS AND DISCUSSION

Inhibitory Concentration 50% (IC_{50})

MTT method was used to determine cell viability after incubation for 24 h. In every treatment EAF and doxorubicin was shown to inhibit cells growth. The IC_{50} value of EEZ for 1st and 6th treatment of DOX was 48.760 $\mu\text{g/mL}$ and 50.380 $\mu\text{g/mL}$. The cytotoxicity estimate of natural product is related to content of active compound in these plants including *Zanthoxylum acanthopodium* DC. Flavonoids, alkaloid and tannin estimated as active compounds [28]. Doxorubicin is one of chemotherapeutic agent showed strong activity on T47D cell lines with IC_{50} value of 0.203 $\mu\text{g/mL}$ and 2.415 $\mu\text{g/mL}$ for 1st and 6th treatment. T47D cells line underwent resistant to doxorubicin pass through to p53 mutation [29,30]. The IC_{50} value after treatment with 6 times of doxorubicin can be seen in Table 1.

Table 1. IC_{50} value on T47D after treatment with 6 times of doxorubicin

Nilai IC_{50} doksorubisin	R-0	R-2	R-4	R-6
EEABA	0,203	0,865	2,410	2,415
	48,760	-	-	50,380

The known resistance mechanisms particularly of doxorubicin are decreasing the intra cellular level of the drug by over expressing efflux pumps [31] and increasing drug detoxification, by upregulation of metabolism enzymes, e.g. glutathione S-transferase (GST) [32]. Doxorubicin resistant cells show reduced activity of topoisomerase II, a target of doxorubicin [33]. A recent studies showed that EMT and cancer stem cells are also factors causing doxorubicin resistance [34,35]. Flavonoid and alkaloid can inhibit over expression of Pgp and modulation of p53 expression (quercetin, fisetin, naringenin, kamferol, berberine, and etc) [36].

CONCLUSION

According to the result obtained, EEZ is potential to defeat chemoresistance on T47D cell line.

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