

## Cytotoxicity Activity of Ethanol Extract of Andaliman Fruits (*Zanthoxylum acanthopodium* DC.) towards 4T1 Breast Cancer Cells

Rosidah<sup>1</sup>, Poppy Anjelisa Zaitun Hasibuan<sup>1\*</sup>, Ginda Haro<sup>2</sup>, Denny Satria<sup>3</sup>

<sup>1</sup>Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155

<sup>2</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155

<sup>3</sup>Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155

**Abstract.** Breast cancer is one of the world's leading cause of death in women. Due to the resistance of chemotherapeutic agents, there is a continuous need to search of natural products with anticancer activity. The use of natural products is expected to increase the effectiveness and decrease side effect. The purpose of this study was to investigate the anticancer activity of ethanol extract of andaliman fruits (EEAF) towards 4T1 cells. Extracts were prepared by maceration using solvent ethanol 96%. 4T1 cells were grown in culture medium DMEM then given by EEAF and doxorubicin. Cytotoxic test in vitro was done by MTT method [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] which is then analyzed using SPSS 21. The results from this study showed that the cytotoxic results (IC<sub>50</sub>) after treatment with EEAF and doxorubicin were 54.48 ± 0.22 µg/mL dan 0.80 ± 0.02 µg/mL. Based on the result above, we conclude that EEAF has cytotoxic activity towards 4T1 cancer cells.

**Keywords:** andaliman fruits, *Zanthoxylum acanthopodium* DC., ethanol extract, breast cancer, 4T1 cell line.

**Abstrak.** Kanker payudara merupakan salah satu penyebab kematian terbesar di dunia pada wanita. Resistensi agen kemoterapi menyebabkan perlu dilakukannya pencarian bahan alam dengan aktivitas antikanker. Penggunaan bahan alam diharapkan dapat meningkatkan efektivitas dan menurunkan efek samping. Tujuan penelitian ini untuk mengetahui aktivitas antikanker ekstrak etanol buah andaliman (EEBA) terhadap sel 4T1. Ekstrak diperoleh melalui maserasi dengan menggunakan pelarut etanol 96%. Sel 4T1 dibiakkan dalam media kultur DMEM kemudian diberi EEBA dan doksorubisin sebagai kontrol positif. Pengujian sitotoksik secara in vitro menggunakan metode MTT [3-(4,5-dimetiltiazol-2-il)-2,5 difeniltetrazolium bromida] yang kemudian dianalisis menggunakan SPSS 21. Hasil uji sitotoksik (IC<sub>50</sub>) yang diperoleh setelah pemberian EEBA dan doksorubisin sebesar 54,48 ± 0,22 µg/mL dan 0,80 ± 0,02 µg/mL. Berdasarkan hasil diatas, dapat disimpulkan bahwa EEBA memiliki aktivitas sitotoksik terhadap sel kanker 4T1.

**Kata kunci:** buah andaliman, *Zanthoxylum acanthopodium* DC., ekstrak etanol, kanker payudara, sel 4T1

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\*Corresponding author at: Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan

E-mail address: poppyanjelisa@usu.ac.id

## 1. Introduction

The world health organization (WHO) reported that breast cancer is one of the leading cause of death and the most common cancer type amongst women worldwide in 2012 [1]. Moreover, breast cancer ranks as the fifth cause of death from cancer overall (522,000 deaths), is the most frequent cause of cancer death in women in less developed countries (324,000 deaths, 14.3% of total), and the second cause of cancer death in developed countries (198,000 deaths, 15.4%) after lung cancer [2]. The diversity of medicinal plants in Indonesia is one of chances in potential development of Indonesia in the globalization era [3], [4]. The use of medicinal plant extracts for the treatment of human disease is an ancient practice and thus has greatly increased in recent years.

Traditionally, andaliman fruits (*Zanthoxylum acanthopodium* DC.) has been used as aromaticum substances, tonicum, and treat dysentery. Indian people have used andaliman to treat paralyzed and skin disease such as abscess and leprosy. Andaliman has been used as spices at North Sumatera especially at North Tapanuli [5], [6], [7]. The plants from *Zanthoxylum* genus contain many compounds such as phenol hydroquinones, flavonoids, steroids/triterpenoids, tannins, glycosides, volatile oils, alkaloids, coumarines, lignans, amides and terpenes [8], [9], [10], [11], [12], [13], [14], [15]. Ethylacetate extract of andaliman fruits (EEA) was showed to have cytotoxicity effect against MCF-7 and T47D cell lines. EEA was found to have synergistic effect when combined with doxorubicin. EEA was showed to have anticancer activity towards mices induced with benzo(a)pyrene, having cardioprotective effect and active on T47D resistance cells [16], [17], [18].

The aim of this study was to determine cytotoxicity activity of ethanol extract of *Zanthoxylum acanthopodium* DC. fruits on 4T1 cells.

## 2. Materials and Methods

### 2.1 Materials

Fresh fruits of *Zanthoxylum acanthopodium* DC. was 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 with number of 332/IPH.1.01/If.07/II/2016, DMSO (Merck), [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT) (Sigma).

## 2.2 Preparation of ethanol extract

The air-dried and powdered fruits of *Zanthoxylum acanthopodium* DC. (1 kg) was extracted by cold maceration with ethanol 96% (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 [4], [19].

## 2.3 Cytotoxicity assay

EEAF was submitted for cytotoxicity test. In that way, 4T1 cell line was grown in DMEM 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<sub>2</sub>) at 37°C. The inoculums seeded at 1 x 10<sup>4</sup> cells/mL at an optimal volume of 0.1 mL per well. After 24 h incubation, the medium was discharged and treated by EEAF. After incubation for 24 h, the cells were incubated with 0.5 mg/mL MTT for 4 h at 37°C. Viable cells reacted 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 microplate reader at λ 595 nm. The data which were absorbed from each well were converted to percentage of viable cells [19], [20].

The equation to determine viability of cells:

$$Viability = \frac{Abs\ of\ treatment - Abs\ of\ medium}{Abs\ of\ control\ cells - Abs\ of\ medium} \times 100\%$$

## 3. Results and Discussion

### 3.1 Cytotoxicity Effect of EEAF

This research was aimed to investigate of EEAF for its cytotoxicity effect on 4T1 cell lines. MTT method was used to determine cell viability after incubation for 24 h. In the treatment was showed the inhibit of cells growth. The IC<sub>50</sub> value was shown on Table 1.

**Table 1.** The IC<sub>50</sub> value of EEAF and doxorubicin

Treatment	Nilai IC <sub>50</sub> (µg/mL)
EEAF	54.48 ± 0.22
Doxorubicin	0.80 ± 0.02

Based on the result from statistical analysis using SPSS 21 the IC<sub>50</sub> value of EEAF towards 4T1 cell was 54.48 ± 0.22 µg/mL and doxorubicin 0.80 ± 0.02 µg/mL. The active extract should have IC<sub>50</sub> value in range 10 - 100 µg/mL [21]. The cytotoxicity estimate of natural product is related to content of active compounds in these plants including *Zanthoxylum acanthopodium* DC. Flavonoids, alkaloids, saponins and tannins estimated as active compounds [16], [22]. This

extract could be developed into co-chemotherapy agent which using together with conventional chemotherapy agents. Doxorubicin has cytotoxicity activity with inhibits synthesis of DNA and RNA through topoisomerase II. In clinical doxorubicin is effective to treat breast cancer patients with metastatic [23].

#### 4. Conclusion

The results reveal that ethanol extract of *Zanthoxylum acanthopodium* DC. fruit potential to treat breast cancer.

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