The Cytotoxic Effect of Ethanolic Extract of *Dasun Tunggal* and Garlic (*Allium sativum*) on Raw 264.7 Cells

Irene Puspa Dewi[1,2], Fatma Sri Wahyuni[3], Yufri Aldi[3], Dachriyanus[3*]
[1]*Akademi Farmasi Prayoga, Jl. Jend Sudirman No 50, Padang, Indonesia, 25129*
[2] *Post Graduate Student, Faculty of Pharmacy, Andalas University, Padang, Indonesia, 25163*
[3]*Faculty of Pharmacy, Andalas University, Padang, Indonesia, 25163*
[*]*Corresponding author. Email: dachriyanus@ffarmasi.unand*

ABSTRACT

Traditionally, *dasun tunggal* and garlic (*Allium sativum*) have been widely used as immunomodulators. Therefore, scientific research is needed to support this traditional use. The first step that needs to be done is to see the cytotoxic effect of the ethanol extract of *dasun tunggal* and garlic on raw 264.7 cells. The method used is the MTT method (3-(4,5,-dimethylthiazol-2yl-2,5- diphenyl-tetrazolium bromide). Raw 264.7 cells were cultured on 96 well plates treated with ethanol extract of *dasun tunggal* and garlic with concentrations of 100, 10, 1 and 0.1 µg/ml. Cells were incubated for 48 hours at 37 ºC, 5% CO2 and then given 100 µl MTT solution 0,5 mg/ml in PBS (Phosphate Buffer Saline) for 4 hours. Formazan crystals formed were dissolved with 100 µl DMSO and absorbency was measured using a microplate reader. Cell viability was obtained in the *dasun tunggal* ethanol extract group, respectively, at concentrations of 100; 10; 1 and 0.1 µg/ml were 103.9, 107.6, 114.2, 122.5 % and in the garlic ethanol extract group 124.2, 122.5, 123.6, 121.7 %. The results in both groups were >100%, meaning that the ethanol extract of *dasun tunggal* and garlic does not have a cytotoxic effect on raw 264.7 cells.

Keywords: *dasun tunggal, garlic, Allium sativum, MTT assay, raw 264,7 cells*

1. INTRODUCTION

Garlic is a spice that grows throughout the year and is widely used in traditional medicine in Indonesia. Garlic attracts researchers interested in studying its properties, notably as an immunomodulator, lowering diabetes and cardiovascular risk, anti-microbial, antifungal, and anticancer because of the chemicals it contains. Garlic contains the organosulfur chemicals aliin (S-allyl-L cysteine sulfoxide) and allicin are responsible for garlic's bioactivity [1].

Dasun tunggal is another variety of garlic. *Dasun tunggal* is a garlic that only produces one clove due to its severe environmental circumstances. *Dasun tunggal* is a native Indonesian plant that is commonly used in

herbal therapy [2]. In general, the chemical compound content of *dasun tunggal* is almost the same as garlic.

One of the methods commonly used to determine cell viability is the MTT (3-(4,5,-dimethylthiazol-2yl-2,5–diphenyl-tetrazolium bromide) method. The MTT method is a simple and convenient method for determining the rate of cell activation and cell growth. Testing with the MTT method can also be used to determine cell proliferation using the colorimetric principle [3] [4].

The principle of the MTT method is to determine the capacity of the mitochondrial enzyme, namely succinate dehydrogenase, which can convert MTT tetrazolium into blue crystals that are proportional to the number of living cells. The MTT method is commonly used to determine the toxicity of a compound to cells, so it can be the basis for determining the concentration of compounds used in subsequent tests [5] [6].

Garlic and *dasun tunggal* are traditionally used as immunomodulators must be supported by scientific proofs. The cell viability testing is required as the first step in a series of tests to determine the activity of garlic and *dasun tunggal*. The goal was to test the cytotoxicity of garlic ethanol extract and *dasun tunggal* on raw cells as a measure of immunomodulatory activity.

2. METHODS

2.1 Chemicals and reagents

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide) was purchased from Sigma, and raw 264.7 cell was obtained from American Type Culture Collection. Gibco provided FBS (Fetal Bovine Serum), Penicillin-Streptomycin, DMEM (*Dulbecco's Modified Eagle Medium*) and Vivantis provided DMSO (Dimethyl Sulfoxide).

2.2. Plant Material

Dasun tunggal and garlic were purchased in Alahan Panjang, West Sumatra. Plants were washed and chopped into small pieces (3-5 mm thickness), then were air- dried in the shade. *Simplicia* is made from dried plant materials that have been pulverized.

2.3. Extraction

Dasun tunggal and garlic *simplicia* were extracted for three days in distilled ethanol. A rotary evaporator was used to evaporate the macerate, yielding a thick extract.

2.4. Cell Culture

Raw 264.7 cells were grown for 24 hours on 96 well plates in DMEM culture media supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C, 5% CO2. At the concentrations of 0.1, 1, 10, and 100 µg/ml, raw cells were treated with ethanol extract of garlic and *dasun tunggal*. Raw cells 264.7 were cultivated for 48 hours at 37 º C with 5% CO2.

2.5 Cell Viability Assay

The medium was removed and cleaned with PBS after 48 hours of incubation. In a 100 µl solution, add MTT 0.5 mg/ml and incubate for 4 hours at 37ºC with 5% CO2. The formazan crystals generated were dissolved in
100 µl DMSO after the MTT solution was discarded. A microplate reader with a wavelength of 595 nm was used to measure the purple hue formed [4]. The following formula can be used to calculate the cell viability :

$$cell\ viability = \frac{cell\ abs\ with\ treatment - cell\ abs\ medium}{cell\ abs\ solvent - cell\ abs\ medium} x\ 100\%$$

3. RESULTS AND DISCUSSION

Cell viability testing with the MTT method uses a colorimeter basis and is the most often used by researchers. This method is intended to check the safety of drugs, both compounds derived from nature and synthetic compounds [7]. In the MTT method, there is a linear relationship between the metabolic activity of cells and the color formed, so that an accurate measurement is obtained in the evaluation of cell viability. The healthy cells will form formazan salts quickly, but dead or inactive cells are unable to do so [8].

This is the first in a series of studies aimed at identifying active compounds in Sumatran herbal medicines that can be used as immunomodulators. By testing the cytotoxicity of raw cells using the MTT method, it can be seen toxicity medicinal plants to raw cells [9]. Doses with cell viability greater than 100 percent can be used for further bioactivity testing [9] [10].

Figure 1 shows the outcomes of the cell viability test. Figure 2 shows the raw 264.7 cell before treatment and after treatment of *dasun tunggal* ethanol extract.

The cell viability of *dasun tunggal* ethanol extract and garlic at concentrations of 0.1; 1; 10; 100 µg/ml was determined to be > 100% based on the findings of cell viability testing of *dasun tunggal* and garlic ethanol extract. As a result, the ethanol extract of *dasun tunggal* and garlic is not hazardous to raw cells, and a dose range of 0.1 – 100 µg/ml can be used for further research. The average cell viability of the ethanol extracts of solitary *dasun tunggal* and garlic were statistically indistinguishable.

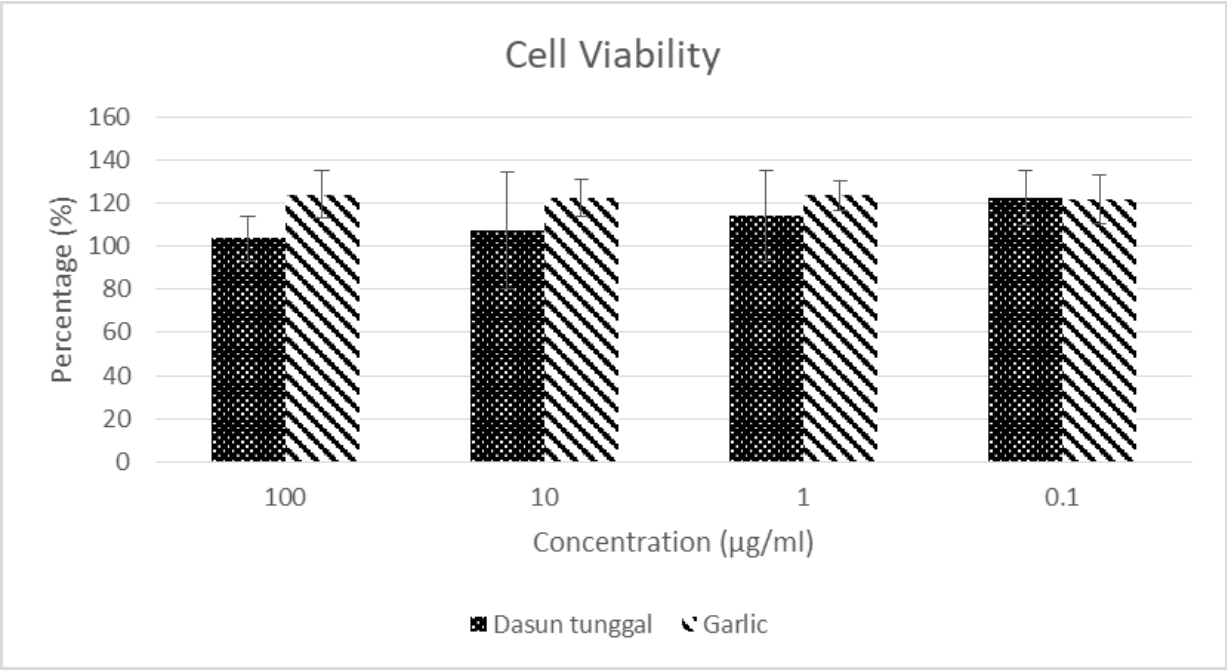
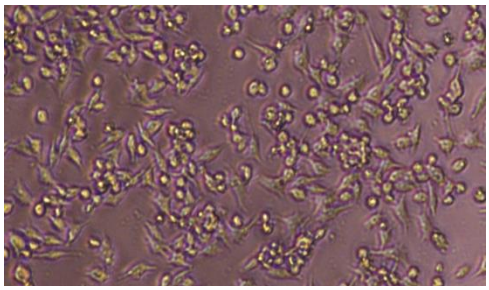
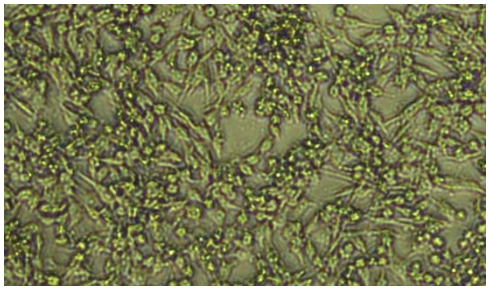


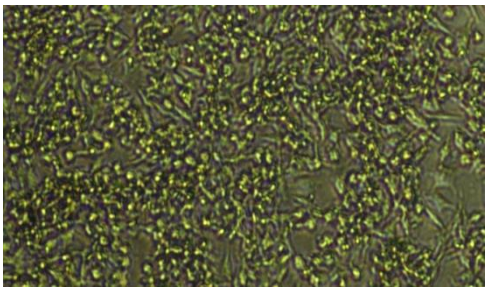
Figure 1: *Dasun Tunggal* and Garlic Ethanol Extract Cell Viability



(A)



(B)



(C)

Figure 2 : Raw 264.7 cell

(A) Before treatment
(B) After treatment with Dasun Tunggal Ethanol Extract
(C) After treatment with Garlic Ethanol Extract

4. CONCLUSION

The ethanol extract of dasun tunggal and garlic at dose 0.1 – 100 µg/ml is not hazardous to raw 264.7 cells.

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