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Original Article

Cytotoxicity Study of Ethanol Extract of Bintangor Leaf (*Calophyllum soulattri Burm.f*) on T47D Breast Cancer Cell Line (Cytotoxicity Study with MTT Assay Method)

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ABSTRACT

Introduction: The public has used Bintangor leaf (*Calophyllum soulattri Burm.f*) for various medical treatments, including treated inflamed eyes and gout. **Aim:** This research aimed to determine the cytotoxic effect of ethanol extract and fraction of *Calophyllum soulattri Burm. f* leaf toward $T_{47}D$ breast cancer cell. **Methods:** The test used $T_{47}D$ breast cancer cells, the 3-4,5-dimethylthiazol-2yl -2,5-diphenyltetrazolium bromide (MTT) test method, and ELISA Reader to determine the absorbance. This method's principle was the presence of tetrazolium salts by the reductase system in the mitochondria of living cells formed purple formazan crystals. The used parameter was the value of IC_{50}. **Results:** The result showed that ethanol extract, n-hexane fraction, ethyl acetate fraction, and butanol fraction did not have a cytotoxic effect on $T_{47}D$ breast cancer cell. The values of IC_{50} respectively are 585.31 µg/ml; 409.33 µg/ml; 534. 08 µg/ml; and 563.22 µg/ml. **Conclusion:** Ethanol extract and *Calophyllum soulattri Burm.f* leaf fraction did not have a cytotoxic effect on $T_{47}D$ breast cancer cells.

Key words: Bintangor Leaf, Breast Cancer Line, *Calophyllum soulattri Burm.f*, Cytotoxicity, MTT Assay, $T_{47}D$.

INTRODUCTION

Indonesia is an island country located in the equatorial zone and known as a country that has natural wealth with a lot of diversity of plants. With the richness of this plant, it supports the development of new drugs from plants. Various types of plants have been widely used for generations to prevent and treat diseases, including cancer treatment. One cancer drug obtained from plants is vinblastine obtained from Vinca rosea, podophyllin obtained from the plant roots of *Podophyllum peltatum*, and paclitaxel that brought from the skin of the pine tree *Taxus breviflora*[1].

The ideal anticancer drugs should be able to counteract cancer cells without damaging normal tissues. However, the current agents did not meet these criteria[2]. Therefore, the research should be continued to find the ideal cancer drug. One of the plants that have anticancer activity is bintangor (*Calophyllum soulari Burm.f*). It is used to wash the inflamed eyes; a decoction of the bark is used to treat vaginal discharge and rheumatism. The seeds of Bintagor are used to treat scabies, ulcers, and hair growers[3].

Some compounds that have been isolated from *Calophyllum soulattri* are terpenoid derivatives called soulattron A[4] and friederin[5]. Xanthone derivatives include soulattrin, caloxanthon B, caloxanthon C, macluraxanthone philattrin, bracixanthon, trapezipholixanthon, and ananixanthons[5]. The xanton literature study shows pharmacological activity as an anticancer characterized by cytotoxic activity and its ability as an antioxidant associated with heterocyclic forms of these compounds. Some heterocyclic compounds have been shown as an anticancer, especially in inducing apoptosis[6,7].

In a previous study, cytotoxic tests of *Calophyllum soulattri* plants were carried out on 4 types of cancer cells, namely HeLa cells (cervical cancer), MDA-MB-231 cells (breast cancer), LS174T (colon cancer), T98G (glioblastoma) and also tested on normal human cells HEK293 (human embryonic kidney cells). The researcher used dichloromethane, ethyl acetate, and methanol as solvents, where the value of IC_{50} on MDA-MB-231 breast cancer cells fulfills cytotoxic requirements with ethanol solvents.

This researcher also tested the pure compounds in *Calophyllum soulattri*, which is friedelin, showing IC value $_{50}$ good against HeLa cells and T98G that is smaller than 30 µg/ml[8].

Based on the research, there was no cytotoxic assay study of ethanol extract of *Calophyllum soulattri* leaves against $T_{47}D$ breast cancer cells. Research on breast cancer cells is essential because breast cancer is one of the biggest cancer killers due to cancer each year[9]. Handling of cancer, in general, is still dependent on chemotherapy derived from synthetic chemicals. However, these chemical compounds can cause multidrug resistance effects, a phenomenon in which cancer cells treated with certain drugs will become resistant to other drugs that have almost the same structure and mechanism of action[10]. However, chemotherapy affecting cancer cells can also affect normal cells that grow rapidly[1].

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In this study, cytotoxic ethanol extract, n-hexane fraction, ethyl acetate fraction and butanol fraction of *Callophylum soulatri Burm* f. leaf were carried out. for $T_{47}D$ breast cancer cells using the MTT Assay method. The parameter observed is the value of IC_{50}. The purpose of this study was to determine the cytotoxic effects of ethanol extract and the leaf fraction of bintangor (*Callophylum soulatri Burm.* f) on $T_{47}D$ breast cancer cells.

MATERIAL AND METHOD

Material

The materials used for the cytotoxicity test were ethanol extract, n-hexane fraction, ethyl acetate fraction, and butanol fraction, leaves of *Calophyllum soulattri Burm*.f obtained from previous researchers at the Faculty of Pharmacy, Universitas Andalas. Breast cancer cells obtained from the Laboratory of Parasitology, Faculty of Medicine, Universitas Gadjah Mada.

The chemicals used are dimethyl sulfoxide (DMSO) (Sigma®), 70% alcohol, the Rosewell Park Memorial Institute (RPMI) medium, trypsin-EDTA (Sigma®), phosphate buffer saline (PBS) (Sigma®), trypan blue and MTT reagent 3-(4,5-dimethylthiazol-2-il) -2,5-diphenyltetrazolium bromide (Sigma®), reagent stopper.

Cell sub-culture

The medium in the flask was discarded, then add 2 ml FBS and stir slowly for five minutes. Then FBS is removed and observed under a microscope. Then add 2 ml of trypsin-EDTA then stir slowly, incubate for 5-10 minutes at 37°C, 5% CO^2, then observe the cell under the microscope. Cells that are ready for use will float and separate from the colon. Then the trypsin-EDTA solution containing the cell was centrifuged at 3000 rpm for 5 minutes. Remove the supernatant, and the pellets were cultivated in 3 mediums. Put in a new flask, stirring gently. Incubation at 37°C, 5% CO_2.

Calculation of number of cell

The flask medium is discarded. A total of 2 ml of trypsin-EDTA is added to the flask, incubated for 5-10 minutes. Then the solution is centrifuged at 3000 rpm and the supernatant is removed. 5 ml of medium was added, the solution is resuspended homogeneously. 10 µl of cell suspense is taken, place it on each of the squares cell calculation boxes then calculated under a microscope.

Cell layout

The cell suspension is made in the medium (measured quantity and volume), mixed perfectly. Add 180 suspensions to each well except the well in the first and last columns. The first and last columns are blanks that only contain medium, while the second column is a control that contains cell suspension in the medium. Incubate at 37°C, 5% CO_2 for 24 hours

Stock solution and dilution of the test solution

Weigh the sample to about 10 mg in Eppendorf. The sample is dissolved in 100 µl DMSO for men get the solution concentration of 0.1 mg/µl. Samples must be completely dissolved in DMSO. Create the main solution by piping 50 µl of the stock solution with 995 µl in the Eppendorf tube.

Perform a level dilution by moving the 500µl test solution from the first tube to the second tube. Do the same for the next tube so that a solution will be obtained with a concentration of 500 µg/ml, 250 µg/ml, 125 µg / ml, 62.5 µg/ml and 31.25 µg/ml in each well on the 96 plates well.

Cell proliferation test

Test plates containing cell wells and incubated for 24 hours. Discard the media on the well 96 plates by turning the plate 180°. Samples are inserted on the plate where the test solution is started from the lowest concentration. Transfer 20 µl of the test solution to each test well into the control well enter 180 µl of media, and the blank is only filled with 200 µl of media. The plate is re-incubated for 24 hours in an incubator 37°C, 5% CO_2.

Piped 10µl of MTT mg/ml solution into each well. Incubate for 3-4 hours at 37°C, CO_2. After 3-4 hours, there will be a purple colour from the formal form of the call to prayer formed. Added 100µl reagent stopper in each well. The plate is wrapped using paper or aluminium foil, leave it for 24 hours at room temperature then read absorption used ELISA reader

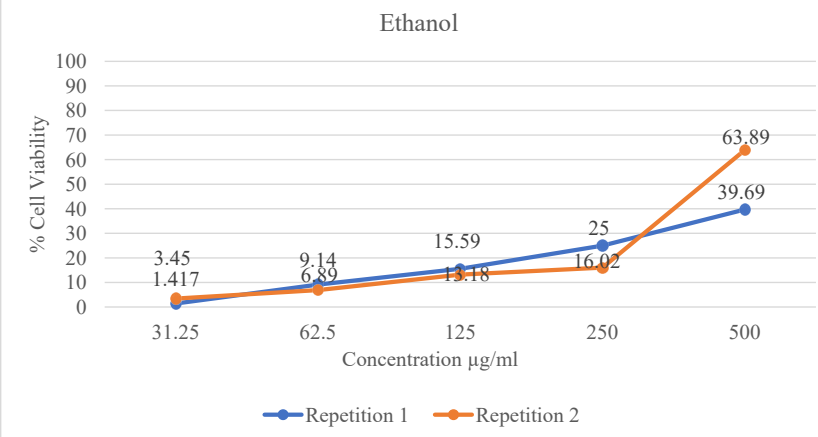

Figure 1: Graph of the relationship between concentrations of ethanol extract of *Calophyllum soulattri Burm*.f with % viability of $T_{47}D$ breast cancer cell.

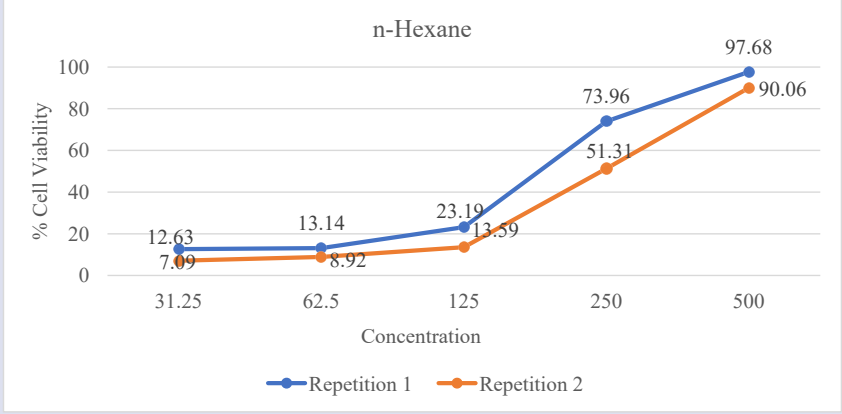

Figure 2: Graph of the relationship between the concentration of n-hexane fraction of *Calophyllum soulattri Burm.f* with% viability of $T_{47}D$ breast cancer cells.

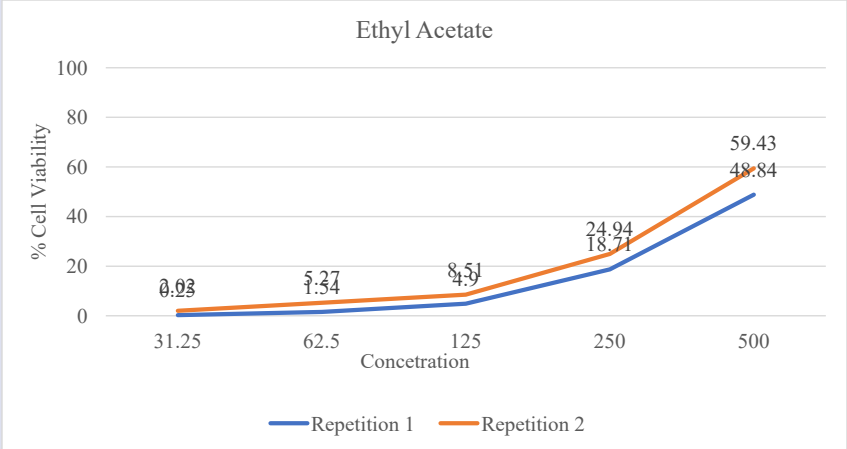

Figure 3: Graph the relationship between the concentration of ethyl acetate fraction of *Calophyllum soulattri Burm.f* with% viability of $T_{47}D$ breast cancer cells.

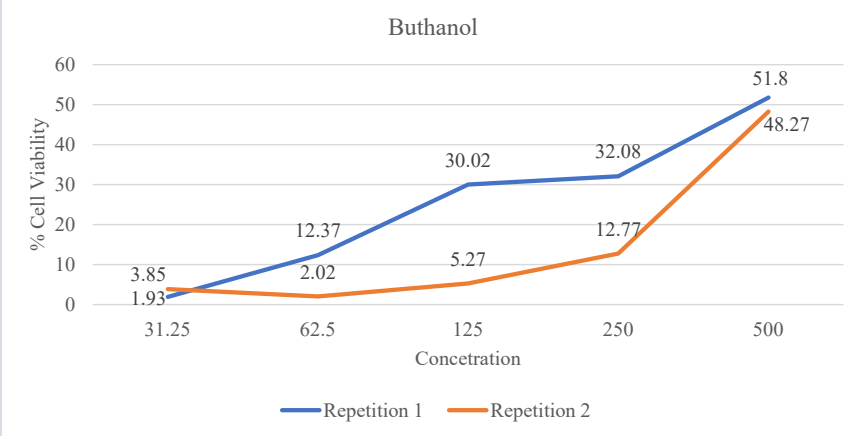

Figure 4: The graph of the relationship between the concentration of butanol fraction of *Calophyllum soulattri Burm.f* with% viability of $T_{47}D$ breast cancer cells.

Analysis of data

By using absorbance data obtained from measurements, it can be determined the percentage of cells inhibited using the following formula[11]:

$$\%\ of\ cell\ viabilty = \frac{A_{\text{treatment}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}} \times 100\%$$

*A = Absorbance

The relationship between the concentration of the test solution and cell viability can be displayed in the form of a concentration chart with live cell presentation. From the graph, the price of IC_{50} can be determined from each solution.

RESULTS AND DISCUSSION

The cytotoxic effect of (*Calophyllum soulatri Burm*. f) fraction was conducted on $T_{47}D$ breast cancer cells using the MTT assay method obtain IC_{50} values of ethanol extract 585.31 µg/ml, a fraction of n-hexane 409.33 µg/ml, ethyl acetate fraction 534.08 µg/ml and butanol fraction 563.22 µg/ml.

DISCUSSION

The MTT method is one of the colourimetric methods using a colour reaction in the measurement of a number of living cells based on changes in solution 3- (4,5-dimethylthiazole-2-il) -2,5-diphenyltetrazolim bromide (MTT) which is a formazan crystal which purple by mitochondria which are active in living cells. MTT is absorbed into living cells and broken down through a reduction reaction by the reductase enzyme in the mitochondrial respiration chain to formazan which is insoluble in water[12].

The parameters used for cytotoxic tests are IC_{50} values. The value of IC_{50} is the concentration of a substance where the substance can inhibit half of the activity or cell growth tested. The IC_{50} value indicates a concentration value that can inhibit 50% of cell proliferation and points to the potential for toxicity of a compound to cells. The greater the price of IC_{50}, the more the compound has no potential as a cytotoxic compound.

According to The American National Cancer Institute, the compound is said to have cytotoxic activity if the IC value is $_{50}$ <30 µg/ml[13]. The MMT assay has been done and the result showed that the IC_{50} of ethanol extract was 585.31 µg/ml, n-hexane fraction was 409.33 µg/ml, ethyl acetate fraction was 534.08 µg/ml and butanol fraction was 563.22 µg/ml. Thus, the ethanol extract, n-hexane fraction, ethyl acetate fraction and butanol fraction have no cytotoxic activity because of the value of IC_{50} was more than 100 µg/ml.

In a previous study, cytotoxic testing of *Calophyllum soulattri Burm*.f on MDA-MB-231 breast cancer cells showed a cytotoxic effect. The extract used in the study used ethanol and methanol solvents[8]. In these studies, conducted using $T_{47}D$ breast cancer cells showed a non-toxic effect. It occurs because of the expression of receptors in different cells. Where prescription expression on MDA-MB-231 cells is epidermal growth factor (EGF) which can change growth factor alpha (TGF), whereas in $T_{47}D$ cells expression of receptors can express positive estrogen and progesterone receptors[13,14]. Progesterone receptors are receptors that can stimulate growth, differentiation, and development of breast cancer. Where excessive exposure to these receptors can cause breast cancer[15]. It is suspected that $T_{47}D$ breast cancer cells given treatment with *Calophyllum soulattri Burm*.f extract cannot occupy and interact with estrogen and progesterone receptors so that it does not cause any effect.

Based on the results of the research that has been done, it can be concluded that the ethanol extract, n-hexane fraction, ethyl acetate and butanol do not have cytotoxic activity. Therefore, further research is still needed by carrying out cytotoxic testing of bintangor leaf extract on other cancer cells.

CONCLUSION

The IC_{50} of ethanol extract was 585.31 µg/ml, n-hexane fraction was 409.33 µg/ml, ethyl acetate fraction was 534.08 µg/ml and butanol fraction was 563.22 µg/ml. Thus, the ethanol extract, n-hexane fraction, ethyl acetate fraction and butanol fraction have no cytotoxic activity because of the value of IC_{50} was more than 100 µg/ml. Based on that results ethanol extract and bintangor leaf fraction (*Calophyllum soulattri Burm. f*) do not have a cytotoxic effect on $T_{47}D$ breast cancer cells. This solutions should be tested on another cancer cell to determine the cytotoxic effect of extract of this plant.

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CONFLICTS OF INTEREST

The authors declare that there is no conflicts of interest regarding the publication of this article.

ABBREVIATIONS

EGF: Epidermal growth factor; HeLa Cell: One of the cells grown from the cervical cancer of a young African-American woman, The designation "HeLa" was taken from the name of Henrietta Lacks; $IC_{50:}$ Inhibitory Concentration; MDA-MB: stands for "M.D. Anderson and MB stands for Metastasis Breast cancer; MTT Assay: Microculture tetrazolium; PBS: Phosphate Buffer Saline; TGF: Tumor Growth Factors, SDS: Sodium dodecylsulphate.

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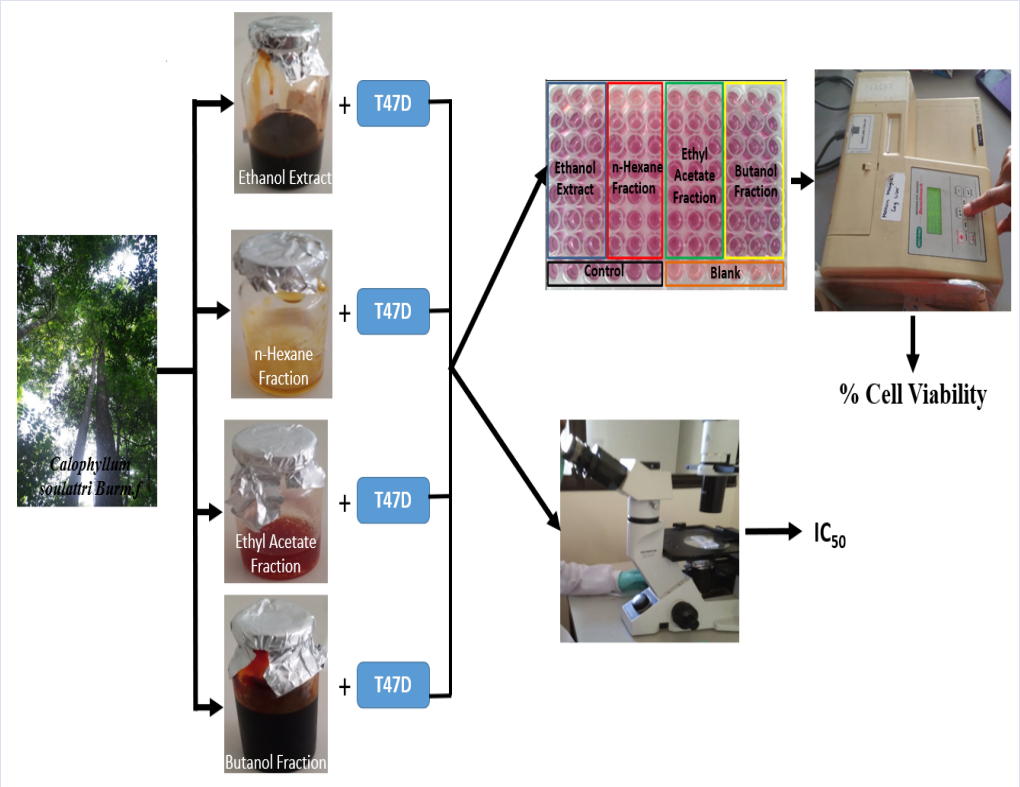
GRAPHICAL ABSTRACT



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