

## Cytotoxicity of Kelakai (*Stenochlaena palustris*) Extract to MCF-7 Breast Cancer Cell

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Article info	Abstract
<b>History</b> Submission: 17-12-2019 Review: 30-05-2020 Accepted: 26-08-2020  <b>*Email:</b> <a href="mailto:harlyanti@poltekkes-palangkaraya.ac.id">harlyanti@poltekkes-palangkaraya.ac.id</a>  DOI: 10.33096/jffi.v7i3.590  <b>Keywords:</b> Kelakai, <i>Stenochlaena palustris</i> , breast cancer, MCF-7, MTT	<i>Breast cancer is reported to rank fifth among all types of cancer with a case of death of 6.6%. In the Central Kalimantan region, early examinations of cancers including breast cancer were carried out and 247 tumors in the breast were identified (1.76%). Kelakai (<i>Stenochlaena palustris</i>) as one of the typical plants of Kalimantan which contain natural chemical constituents has been reported to be effective as an anti-inflammatory and antioxidant, so that with this potential can be developed to overcome diseases associated with it, one of them is breast cancer. This study aimed to examine the cytotoxicity of MCF-7 breast cancer cells using ethanol extract. Identification of chemical constituent by the qualitative method using color reagents. The cytotoxicity assay of kelakai extract against MCF-7 cells conducted in vitro by the MTT reduction method. The variation of concentration used is 1000; 500; 250; 125; 62.5; 31.5; and 15.625 µg/ml, doxorubicin as a positive control was performed in a concentration of 1 µg / ml. The results of the cytotoxicity assay showed that the kelakai extract had a toxic effect on MCF-7 cells with an IC<sub>50</sub> value of 493.57 µg / ml.</i>

### I. Introduction

Cancer itself has been a serious problem in developed country as well as developing country. Cancer incidence has decreased in 2018 reached 9.6 million cases, with 8.8 million cases cause death. Breast cancer was reported occupy the fifth order among all types of cancer with death cases as many as 627000 (6.6%). It increases compare to previous data stated that breast cancer occupy the sixth order (WHO, 2018). In Centre Borneo, it has been conducted early assessment to cancer particularly cervical and breast cancer. Tests conducted in women aged 30 – 50 years old in 2017 known as tumor/bump in breast as many as 247 patients (1.76%). This score is more abundant than last year in 2016 as many as 82 patients (Dinas Kesehatan, 2018).

Kalimantan is an island in Indonesia which is known with its biodiversity. Besides, knowledge about traditional medicine has been passing down from one generation to other generation in Kalimantan native ethnicity (Fahruni, et al., 2018). One of the typical plants of Kalimantan which has a specific history as traditional medicine is kelakai. Kelakai is very commonly consumed by the community and easily to find around the house. Kelakai has been reported to be effective for treating anti-inflammatory. The alkaloid content can inhibit prostaglandin synthesis and steroid content can inhibit the activity of phospholipase and change arachidonic acid into prostaglandin, and inhibit cytokine production. In other studies, Kelakai is

reported potent to TNF- $\alpha$  levels thus can be developed as an anti-malaria drug (Margono et al., 2016).

The Dayak tribes believe that by consuming the leaves of kelakai, they can treat various diseases and for breastfeeding mother the leaves of kelakai is believed to increase and expedite the production of breast milk. This is related to the efficacy in fulfilling Fe in nursing mothers and toddlers (Indrayanti et al., 2016). Breastfeeding can be one way that prevent breast cancer because during breastfeeding there are hormonal changes, one of which is a decrease in the hormone estrogen. Estrogen is one of the triggers for breast cancer. At the time of breastfeeding progesterone will be more dominant than the hormone estrogen, thus women can avoid breast cancer.

Based on that, it should be conducted scientific assay to prove cytotoxicity of Kelakai to breast cell cancer MCF 7. The aim of this research is to become a novel new breast cancer medication which can be developed as a traditional breast cancer medicine or active compound isolation which can be a choice of chemotherapy, as well as scientific basis data for further research.

### II. Research Method

#### II.1 Material

Materials used in this research is Kelakai, DMSO, ethanol 96%, *blue tip*, *yellow tip*, Media RPMI, MTT reagent, MCF-7 cell cancer, Doxorubicin, 96 wellplate.



## II.2 Tools

Tools applied are maceration package, stirring rod, extract, measuring glass, test tube, chemical glass, micropipette, tweezers, bath, spoit, incubator, endorf tube, rotavapor, analytical measurement, pipette, microplate reader.

## II.3 Extraction

Powdered plants were extracted by maceration method using 96% ethanol liquid. Initial maceration uses 96% ethanol with comparison between sample and solution 1: 4 for 3 days. The filtered solution is collected and then the solution is re-macerated again with similar liquid. The results of the storage are collected and then evaporated with a rotary evaporator until the extract is obtained.

## II.4 Cytotoxicity Assay

### 1. Extract and Control Sample Preparation

The kelakai plant extract is prepared with a final concentration of 1000; 500; 250; 125; 62.5; 31.5; and 15.625  $\mu\text{g} / \text{ml}$ . The positive control applied in this study was doxorubicin at the final concentration of 1  $\mu\text{g} / \text{ml}$ .

### 2. Activity Assay

Cytotoxicity assay of MCF-7 breast cancer cells was performed using the MTT method in accordance with the modified Graidist (2015) method. MCF-7 breast cancer cells were incubated in 96 wellplates for 24 hours. After that the extract and control were added and incubated for 72 hours. After that the cells were isolated with 1X PBS liquid and incubated with 100  $\mu\text{l}$  of 0.5 mg / ml MTT at 37°C. After 30 minutes, 100  $\mu\text{l}$  of the MDO stopper reagent was added to each wellplate. Absorbance was measured at a wavelength of 550 nm using a microplate reader. The absorbance value obtained is used for the percentage of cell viability calculated by the formula:

$$\% \text{ Viability} = \frac{\text{Cell absorbance with treatment} - \text{absorbance of blank sample}}{\text{Absorbance of control cell} - \text{absorbance of blank sample}} \quad (1)$$

## II.5 Result analysis

The data obtained was tabulated and analyzed using Microsoft Office Excel 2013. The results obtained were translated into the average percent cell viability and standard deviation (SD). After that, the  $\text{IC}_{50}$  determination of the plant extracts was applied.

## III. Result and Discussion

### III.1 Sample preparation

The sample used in this study was a plant that was obtained in Palangka Raya City (Figure 1). The sample used is dried first, after it is dried and then powdered. The sample powder obtained was 1165 g, then extracted.



Figure 1. Kelakai

Extraction is the process of separating compounds from plant parts using selective solvents through standard procedures. The aim is to separate dissolved plant metabolites from insoluble residues. During the extraction process, the solvent will diffuse into the solid material and dissolve the compound with similar polarity. One of the most commonly used extraction methods is maceration. Maceration is the simplest extraction technique which is conducted by immersing plant material (in the rough or powder form) in a container using solvent and left at room temperature for at least 3 days while occasionally stirring. This process is intended to soften and break the plant cell walls to release soluble compounds so that more extraction results can be obtained (Nn, 2015; Tiwari et al., 2011).

The solvent used in this extraction process is 96% ethanol. Ethanol is used as a universal solvent because it is easy to dissolve active compounds both polar, semi-polar and non-polar. In addition, ethanol easily penetrates into plant cells. A total of 1165 g of powdered Kelakai was extracted and 25 g of extract was obtained. The yield values obtained are shown in table 1.

$$\text{Rendamen calculation} = \frac{\text{the weight of extract}}{\text{the weight of powdered}} \times 100\% \quad (2)$$

Table 1. Rendamen value of Kelakai

Sample	Rendemen (%)
Kelakai Extract	1.72

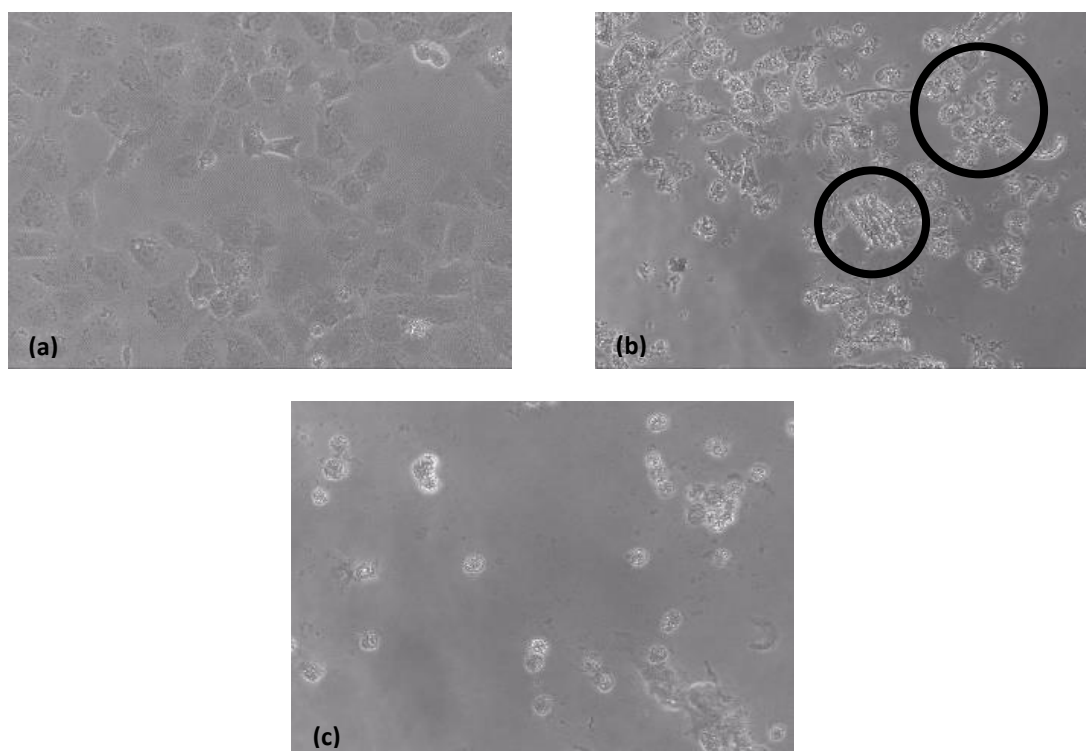
Extract rendamen was calculated to simplisia powder.

### III.2 Cytotoxicity Assay to Breast Cancer Cell MCF-7

MCF-7 cells are human breast adenocarcinoma cells which endogenously express estrogen receptor (ER)  $\alpha$  mainly and also some ER  $\beta$  (Dagher et al., 2012; Vantangoli et al., 2015). MCF-7 cells are estrogen responsive, and are often used in vitro to study estrogen receptors for positive breast cancer. The popularity of MCF-7 is largely due to its extraordinary hormone sensitivity through the expression of estrogen receptors (ER) (Holliday and Speirs, 2011; Vantangoli et al., 2015). In addition to expressing estrogen receptors, MCF-7 cells also express androgen, progesterone, and

glucocorticoid receptors. As a targeted agent for all steroid signaling pathways, these cells are also active for testing against metastatic breast cancer. The cytotoxicity test of the user extracts against

MCF-7 cells was carried out using the MTT test method.

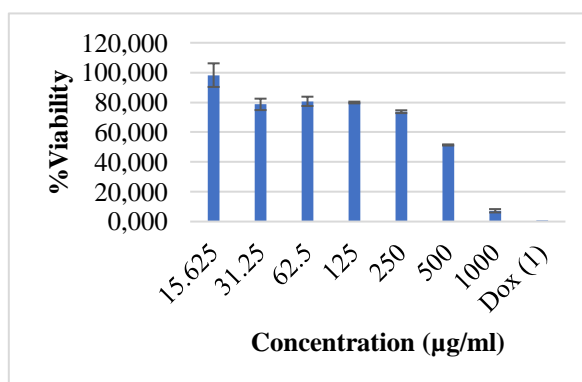


**Figure 2.** MCF-7 Cell growth: (a) Before treatment; (b) After treatment with kelakai extract; and (c) after treatment with doxorubicin

MTT testing is an appropriate and sensitive technique for measuring cell cytotoxicity. This method can be applied for testing cytotoxicity, cell viability, and proliferation in cell biology based on the reduction of tetrazolium salts through dehydrogenase succinate mitochondria in cells (Houdkova et al., 2017; Stockert et al., 2012; Yang et al., 2015). Measurement of cell viability plays an important role in toxicity assay (Xu et al., 2015).

The condition of MCF-7 cells after treatment with the extract is shown in Figure 2b. When compared with the condition of the cells before treatment (Figure 2a), there will be seen many cells that are not intact or die after treatment with the extract. Percentage of MCF-7 cell viability decreased with increasing concentration of the extracts used and there was a difference between the various variations of the concentration used, 15.625; 31.5; 62.5; 125; 250; 500 and 1000  $\mu\text{g} / \text{ml}$  (Figure 2). Various variations of these concentrations, respectively, revealed a value of percent viability that is  $98.27 \pm 7.92$ ;  $78.65 \pm 3.83\%$ ;  $80.69 \pm 3.12\%$ ;  $79.91 \pm 0.59\%$ ;  $73.72 \pm 0.94\%$ ;  $51.40 \pm 0.37\%$  and  $7.24 \pm 1.12\%$  ( $\text{IC}_{50}$ :  $493.57 \mu\text{g} / \text{ml}$ ).  $\text{IC}_{50}$  values obtained  $> 200 \mu\text{g} / \text{ml}$  indicate that the sample activity was very weak (Bahriul et al, 2014). On the other hand, the doxorubicin percent viability

concentration of  $1 \mu\text{g} / \text{ml}$  was  $0.82 \pm 0.38\%$  and killed almost all MCF-7 cells (Figure 7c).



**Figure 2.** %viability cell of Kelakai extract in various concentration with positive control of Doxorubicin (average  $\pm$  SD,  $n = 3$ )

According to Adawiyah and Rizki (2018) the antioxidant activity of Kalakai root extract was assayed using the Inhibitory Concentration 50 ( $\text{IC}_{50}$ ) parameter with DPPH (2,2-Diphenyl-1-Pikrihidrazil) method having a very strong antioxidant activity with an  $\text{IC}_{50}$  value of  $19.06 \mu\text{g} / \text{ml}$ . Isolation fraction of ethanol extract and ethyl acetate of the leaves of the leaves revealed the

highest cytotoxicity effect on HeLa cells and the highest free radical inhibitory activity. IC<sub>50</sub> values were 4.58 µg / ml and 8.60 µg / ml, respectively, and the value of free radical inhibition activity was 98.47 ± 0.002% (ED<sub>50</sub> = 0.120 mg / mL) and 81.38 ± 0.018% (ED<sub>50</sub> = 0.650 mg / mL) (Arullappan et al., 2017). If the IC<sub>50</sub> value obtained <50 µg / ml indicates that the sample activity is categorized as very strong (Bahriul et al, 2014)

The bioactive content of the sample is a major factor in potential cytotoxicity and antioxidant agents. Flavonoids have been proven to be a therapeutic agent compound for preventing cancer by their mechanism of action decreasing cancer cell proliferation and significantly reducing the expression of angiogenesis, vascular endothelial growth factor (VEGF), in ovarian cancer cells. Flavonoids have also been shown to inhibit cell proliferation by regulating cyclin-dependent kinase 1 (CDK1) and cyclin B, markers of the transition from G2 to M phases, and through regulating tumor suppressor gene that plays an important role in holding the cell cycle, p53 in breast cancer cells MCF-7 and HeLa cervical cancer cells (Arullappan et al., 2017). Further isolation and purification, as well as the characterization of pure compounds which are potentially needed to lead to more specific bioactivity and to obtain maximum test results.

#### IV. Conclusion

The results of cytotoxicity tests on MCF-7 cells revealed IC<sub>50</sub> values: 493.57 µg / ml, but this value obtained > 200 µg / ml revealed that the sample activity was very weak.

#### V. Acknowledgement

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