

Research Article

**Cytotoxicity Test on Breast Cancer Cell Lines T-47D treated
with *Pisang Kepok* Peel Extract (*Musa balbisiana*)**

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ABSTRACT

Utilization of natural ingredients such as fruit as a breast cancer treatment agent is beginning to be widely studied by the scientists. The goal is to reduce the side effects of chemotherapy agents. One of the potential natural ingredients as anti-cancer is *Pisang Kepok* peel (*Musa balbisiana*). This kind of banana contains various phytochemical compounds and high antioxidant activity that can induce cancer cell apoptosis. The purpose of this research is to know the IC₅₀ value of *Pisang Kepok* peel extract thus can be known its potential as an anticancer agent. The lower of IC₅₀ value is more cytotoxic. Method: This research is experimentally done by using CRD (Completely Randomized Design). The stages of this research are extraction and cytotoxic test (MTT assay). The extraction method was used by maceration (ethanol 95%). The cytotoxic test was performed by giving *Pisang Kepok* unripe peel extract on the T-47D cell lines as well as giving the MTT reagent until altering of colour and followed by adding SDS stopper 10%. Then, read the absorbance with ELISA reader at λ 595 nm and eventually analyzed by SPSS Probit. Conclusions: Based on this results, it can be concluded that *Pisang Kepok* peel extract has moderate cytotoxic to T-47D cell lines that is able to induce the death of cancer cells, therefore indicate its potential as a candidate for breast cancer chemoprevention agents, especially at high concentrations (>250 μ g/mL).

Keywords: breast cancer, chemoprevention agents, *Pisang Kepok* peel, T-47D

Introduction

Cancer is one of the leading causes of death in the world [1,2,3]. Othman [3] suggests that by 2020, cancer deaths are expected to increase by 104%. One of the cancers that cause these deaths is breast cancer [4,3]. Based on the results of the Basic Health Research of 2013 showed that breast cancer occupied the highest prevalence of 28.7% (12,014 people), followed by cervical cancer 10.4% (4342 people) [5]. One of the steps in treating cancer is through chemotherapy. However, this way has side effects, for example nausea, pain, fatigue, and anxiety [6]. Scientists have begun researching the use of natural ingredients such as fruit as a cancer therapy agent or chemoprevention agent to increase the sensitivity of

cancer cells and reduce the side effects of chemotherapy agents [7,8].

The Prophet Muhammad said in a hadith as follows:

لِكُلِّ دَاءٍ دَوَاءٌ، فَإِذَا أَصِيبَ دَوَاءُ الدَّاءِ بَرَأَ بِإِذْنِ اللَّهِ عَزَّ وَجَلَّ

Meaning: "Every disease has a cure. So if the medicine is about the disease will be healed with the permission of Allah AzzawaJala "(HR Muslim No.575).

The hadith explains that every disease can be cured by Allah's permission, including cancer. Free radicals in cancer cells can be resisted with antioxidants. The content of antioxidants in the fruit has the potential as a cancer therapeutic

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agent and to strengthen the scientific truth of the hadith. One of the fruits that contain antioxidants is bananas. Bananas contain antioxidants and high fiber, especially on the peel. The fiber content of banana peel is 50g/100g dry weight [9] and galocatechin compound on the banana peel (Musa Cavendish) 158 mg/100 g dry weight, while in the pulp of 29.6 mg/100g dry weight [10].

High levels of antioxidant activity in banana peels (95.14%) are possible because of phytochemical compounds such as flavonoids (catechins), tannins, terpenoids, alkaloids, saponins, and quinones [10,11,12]. Phytochemical compounds can induce apoptosis, which is a major problem in the growth of cancer cells. One of the models to know this is by *in vitro* test using T-47D cells. T-47D cells are epithelial cells taken from adult female breast tissue affected by *ductal carcinoma*.

Furthermore, those studies support the need for this research to determine the value of IC_{50} extract of banana peel on T-47D cells. The results of this research are expected to provide the potential information of *Pisang Kepok* peel extract as an effective alternative cancer therapy, easy to obtain and can reduce side effects caused by chemotherapy agents.

Material and Methods

Sample Preparation and Extraction

Taken *Pisang Kepok* unripe peel waste from Belahanrejo Village, Kedamean, Gresik, East Java and the plant determination was validated by LIPI (Indonesian Institute of Science) Purwodadi as *Musa balbisiana* (ABB) cv *Pisang Kepok*. Banana peel was collected, washed, drained water, dried, after that small chopped to facilitate the drying process. Drying is done with oven temperature 45°C then mashed to become powder. The extraction is done by maceration, protected from direct sunlight at room temperature. A total of 100 grams of mashed banana peel is macerated with 300 ml of ethanol p.a. The extract obtained was then filtered with filter paper and the Buchner funnel using a vacuum Erlenmeyer. Moreover, the filtrate was evaporated with a rotary vacuum evaporator and then incorporated in a temperature incubator 20°C to obtain a crude extract [12] before stored in the freezer 4°C until ready to use for cytotoxic test.

Cytotoxic Test (CCRC, 2007)

Preparation, Counting Cells, and Laying of Cells on the Plate

T-47D breast cancer cells taken from Gadjah Mada University (UGM) Yogyakarta collection are observed under an inverted microscope and are readily harvested when it is confluent 75-90%. The harvested cells then transferred to the haemocytometer by micropipette were then removed 10 μ L, observed and counted under an inverted microscope. Cells were counted in 4 chambers of haemocytometer. 1,8 mL of cells were laid and 10 mL RPMI-1640 media that supplemented by 10% Fetal Bovine Serum (FBS) and antibiotics was added as calculated to 96-well plates (100 μ l media containing cells for each well) and incubated at least 4 hours at 37 °C in a 5% CO₂ incubator.

Making solutions and Tretaments of Sample to Cells

Samples of banana peel extract were weighed 10 mg and dissolved in 100 μ L DMSO (*dimethyl sulfoxide*). Taken cells from the incubator, observed, then the cell medium is discarded, PBS 100 μ L is inserted into all wells filled with cells and thrown back, sample solution is inserted with concentration series of 1000; 500; 250; 125; 62.5; and 31.25 μ g/ ml and repeated 3x (triplo). Lastly, the cells incubated for 24 hours at least. This is also done for positive control by Doxorubicin with similar concentration.

Giving MTT reagent and Data Analyze

Cell media removed and washed with PBS. A solution of 100 μ L 3-[4,5-dimethyl thiazole-2-yl(-2,5- diphenyltetrazoliumbromide)] MTT (Sigma) that dissolved in PBS as the MTT reagent in the conical was added culture medium up to 10 mL then distributed to each well. Incubate for 2-4 hours in 5% CO₂ incubator at 37°C (until formazan crystals or color change to blue-purple). Added 100 μ L SDS stopper 10% (*Sodium Dodecyl Sulfate*) in 0.1N HCl, then plate wrapped and re-incubated in the dark (room temperature) for one night. The absorbance value reading was done with ELISA reader at $\lambda = 595$ nm. Data from viable cell percentage then analyzed to know IC_{50} value by using SPSS regression analysis (probit / logit) 95% confidence for each concentration.

Results and Discussion

Treatment results showed the effect of giving extract to viable cell percentage. Quantitatively, the effect of banana peel extract on T-47D cells (Table 1) shows that at concentrations of 1000 $\mu\text{g/mL}$ and 500 $\mu\text{g/mL}$, the percentage of viable cells remained only 3.74% and 3.94%. However, at low concentrations there are still many living T-47D cells. This suggests that the increase in sample concentration is inversely proportional to the percentage of viable cells, it means that the higher the sample concentration is given, the lower the viability of the cell (Figure 1). At high concentrations, the extract can induce mortality, but at low concentrations it actually retains cell life.

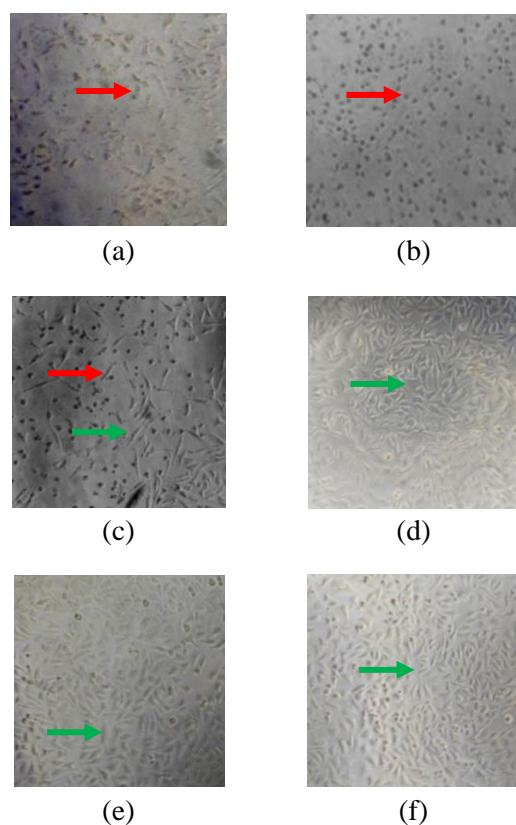
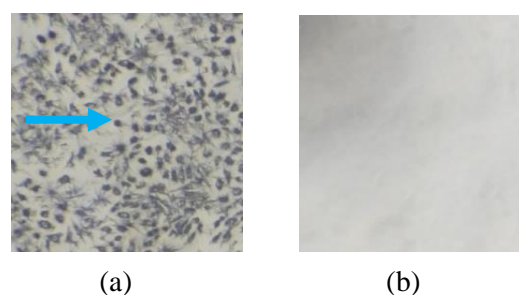


Figure 1 T-47D cells after 1x24 hours were treated with banana peel extract with concentration at a) 1000 $\mu\text{g/mL}$; b) 500 $\mu\text{g/mL}$; c) 250 $\mu\text{g/mL}$; d) 125 $\mu\text{g/mL}$; e) 62.5 $\mu\text{g/mL}$; f) 31.25 $\mu\text{g/mL}$ (100x magnification), green arrow (→) shows live cells while red arrow (→) indicates dead cell.

Table 1. Percentage of T-47D living cells (%) after treated with banana peel extract and doxorubicin (positive control) using MTT assay method

Concentration ($\mu\text{g/mL}$)	Percentage of viable T-47D cell lines (%) at ...	
	Extract	Doxorubicin
1000	3.74	40.72
500	3.94	46.36
250	63.90	54.51
125	81.23	50.42
62.5	91.45	55.47
31.25	91.97	41.47

The effect of banana peel extract was strengthened by observation under an inverted microscope after 4 hours of MTT reagent (colorimetric reaction). In Figure 2 (a) it appears that a lot of formazan crystals are formed to show that many cells are still alive or still doing metabolism because they can reduce MTT salts. Figure (b) shows the media controls that are not contaminated by bacteria or fungi. While in the picture (c) visible levels of formazan formation even less in the picture (d) very little. Based on the formation of formazan crystals and cell density, there was a difference between cell control and T-47D cells treated with banana peel extracts. In addition, the results of treatment with *Pisang Kepok* peel extract also resembles a positive control (T-47D cells that given doxorubicin), which found many dead T-47D cells. These results suggest the same potential of banana peel extracts such as doxorubicin, because it can induce death in T-47D cancer cells so that it cannot reduce MTT salt to formazan crystals.



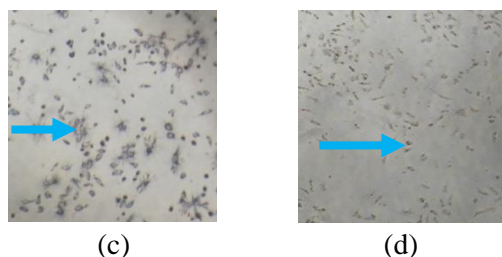


Figure 2 Formazan crystals formed after MTT reagent a) T-47D cell control b) media control c) doxorubicin (positive control) d) treatment of banana peel extract (100x magnification), blue arrows (→) shows formazan crystals

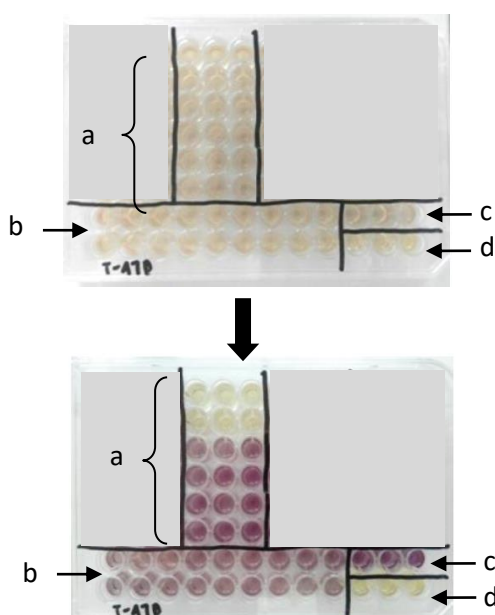


Figure 3 The alteration of colour after giving of MTT reagent and SDS stopper a) extract sample (from higher to lower concentration) b) positive control c) cell control d) media control. The yellow colour shows the dead cells and the purple colour shows the live cells

Visually, the results on 96-well plates after giving of MTT reagent and SDS stopper also showed the effect of giving *Pisang Kepok* peel extract to T-47D cells. The result (Figure 3) shows a yellow colour in some wells of the 96 well plate, precisely at concentrations of 1000 $\mu\text{g/mL}$ and 500 $\mu\text{g/mL}$. Whereas the concentrations of 250 $\mu\text{g/mL}$, 125 $\mu\text{g/mL}$, 62.5 $\mu\text{g/mL}$, and 31.25 $\mu\text{g/mL}$ were changed to purple, this altera-

tion indicates the cells were still alive due to MTT reduction metabolism.

Based on SPSS regression analysis, IC_{50} value obtained from banana peel extract was 220.375 $\mu\text{g} / \text{ml}$, while positive control or Doxorubicin was 33.844 $\mu\text{g} / \text{mL}$ (Table 2). When compared with the positive control, IC_{50} values obtained are high and show banana peel extract is less potential for T-47D breast cancer cell therapy. This is based on The American National Cancer Institute, which states that a crude extract is said to have cytotoxic activity when $\text{IC}_{50} < 20 \mu\text{g} / \text{mL}$ [13].

Table 2. IC_{50} value of each treatment with SPSS Probitanalyze

Treatments	IC_{50} value
<i>Pisang Kepok</i> peel extract	220.375 $\mu\text{g/mL}$
Doxorubicin	33.844 $\mu\text{g/mL}$

When viewed from the percentage of living cells from cancer cells, banana peel extract has the potential at high concentrations to induce the death of T-47D cancer cells. This is seen from the concentrations of 1000 $\mu\text{g/mL}$ and 500 $\mu\text{g/mL}$ that viable cells percentage is only 3.74% and 3.94%.

Based on the cytotoxicity category classification of a sample, potentially cytotoxic when $\text{IC}_{50} < 100 \mu\text{g} / \text{ml}$, moderate cytotoxicity when $100 \mu\text{g} / \text{ml} < \text{IC}_{50} < 1000 \mu\text{g} / \text{ml}$, and not toxic when $> 1000 \mu\text{g} / \text{ml}$. So it can be seen that banana peel extract classified as moderate cytotoxic. The group of compounds from a potentially cytotoxic sample can be used as an anticancer agent whereas moderate cytotoxicity can be used for chemoprevention that can prevent and inhibit the growth of cancer cells [14,15].

Previous research that has been done by Nam (2014) shows that the *Pisang Ambon* (*Musa sapientum*) peel has the potential as chemopreventive agent and co-chemotherapy in breast cancer [16]. This supports the potential of *Pisang Kepok* (*Musa balbisiana*) unripe peel extract as a candidate for the chemo-preventive agent of breast cancer, judging by the higher antioxidant activity held than the *Pisang Ambon* peel. Atun, et al. [17] reported that *Pisang Kepok* peel extract had higher antioxidant activity with ability to inhibit 50%

oxidation at concentration 693,15 mg / ml compared with *Pisang Ambon* peel extract at concentration 5000 mg / ml [18].

Based on cytotoxic test in this study, it can be seen that *Pisang Kepok* peel extract can inhibit 50% growth of T-47D cancer cells at concentrations of 220.375 µg / mL (IC₅₀ value). This suggests that banana peel extract with moderate cytotoxicity potency can be exploited for chemoprevention, whereas for cancer therapeutic agents candidates require high doses. As the word of Allah in QS.Shad 38:37 that everything created by God has its own benefits, including the banana peel.

Conclusion

The result of cytotoxic test (MTT assay) showed IC₅₀ extract of *Pisang Kepok* (*Musa balbisiana*) peel on T-47D breast cancer cells was 220,375 µg / mL, it means moderate cytotoxic. This suggests that banana peel extract is able to induce the death cells on T47D breast cancer cells especially at high doses.

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