

Potential of Pummelo Citrus Fruit Extract (Citrus Maxima), the Magetan Typical Plant as an Anti-Breast Cancer Agent

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Submission date: 21-Dec-2020 07:25PM (UTC-0800)

Submission ID: 1480378419

File name: Jurnal_Aloha_Likopen.docx (46.53K)

Word count: 2409

Character count: 12554

ABSTRACT

Breast cancer is a disease that is quite a prevalence. Based on data from the Ministry of Health, the incidence of breast cancer continues to increase. In 2018 1.79 per 1000 population developed cancer, whereas in 2013 it was only 1.4 per 1000 population. Some of the drugs used in breast cancer chemotherapy are fluorouracil, doxorubicin, and cyclophosphamide. Combination therapy is chosen to accelerate the killing of cancer cells. These drugs generally work by inhibiting fast-growing cells but have a weakness which is not selective, so that not only cancer cells are inhibited, but normal cells with fast growth are also inhibited, such as hair and nail growth. Therefore, it is necessary to develop a selective breast cancer drug.

The pummelo orange may be potential as an anti-breast cancer agent because it contains lycopene compounds. Lycopene works by suppressing cell proliferation or multiplication. In breast cancer patients, cell multiplication occurs very rapidly. Lycopene is also able to slow down or even prevent the oxidation process of other molecules and eliminate free radicals in the body that can cause cell damage. Free radicals can bind to DNA, protein, and lipid which will damage their physiological functions, which in turn can lead to the development of breast cancer. Lycopene is a very effective free radical eliminator. Through these two mechanisms, suppressing cell multiplication and eliminating free radicals, lycopene might be used as a breast cancer therapy

The cytotoxic activity test of pummelo citrus fruit extract was carried out using the MTT method on MCF-7 cells. The pummelo citrus fruit was extracted by maceration method using 96% ethanol as a solvent. Breast cancer cell cultures (MCF-7 cells) were transferred as many as 1×10^4 cells/well in culture media which consisting of FBS, penicillin-streptomycin, amphotericin-B, and RPMI 1640 into 96-well plates and incubated in a 5% CO₂ incubator overnight. Furthermore, the test sample was given with a series of levels and made replications three times (triple), then incubated again overnight. On the third day, we added MTT reagent, and after 4 hours the formazan crystals were formed in the living cells. Furthermore, a SDS stopper was added to stop the MTT reaction. Then, the absorbance reading was carried out using an ELISA reader at a wavelength of 595 nm and continued to determine the value of IC50.

The results showed that the IC50 value of pummelo orange extract was 234 $\mu\text{g}/\mu\text{L}$. It showed that pummelo orange extract had potential as an anti-breast cancer agent. This study could contribute to the development of breast cancer drugs. Pummelo fruit extract which is selective against cancer cells can be the therapy of choice in the treatment of breast cancer patients whose prevalence is high in Indonesia.

Keywords: breast cancer, cytotoxic activity, pummelo, lycopene

INTRODUCTION

Currently, breast cancer is one of the relatively prevalent diseases worldwide. Based on data from the Ministry of Health, the incidence of breast cancer continues to increase. In 2018, 1.79 per 1000 population developed cancer, whereas in 2013 it was only 1.4 per 1000 population. Breast cancer is also a type of disease that has a high mortality rate. This creates a particular concern, especially efforts to treat the disease. Currently, chemotherapy is still an option for breast cancer treatment [1 & 2]

Chemotherapy is a series of therapies commonly used in breast cancer patients. Some of the drugs used in breast cancer chemotherapy are fluorouracil, doxorubicin, and cyclophosphamide. Combination therapy is usually chosen to speed up the killing of cancer cells. The mechanism of these drugs occurs in the metaphase of all cells so that it quickly kills all cells, including normal cells. [3]

Currently, alternative therapies are needed for breast cancer patients. A therapy which can selectively kill breast cancer cells alone, without damaging other cells that have a high growth rate, including hair and nails. One alternative therapy is the use of plants as breast cancer therapy.

One of the plants that may be used as a breast cancer therapy is pummelo (*Citrus maxima*) which is a typical plant in Magetan district. Pummelo, which is abundant in Magetan district, needs to be developed as a potential anti-breast cancer agent. The pummelo orange is potential to develop as an anti-breast cancer agent because it contains phytochemical compounds, one of which is lycopene [4]

Lycopene is one of the free anti-radical compounds found in pummelo (*Citrus maxima*). The lycopene content in pummelo fruit is more than in other parts such as leaves and fruit skin. Lycopene works by suppressing the proliferation or multiplication of cells through mutations in the initiation and progression phase of cancer. In breast cancer patients, cell multiplication occurs quite rapidly, so that with the presence of lycopene, breast cancer cell growth may be inhibited. Lycopene compounds can minimize the oxidation process and reduce the presence of free radicals in the body. Free radical compounds interact with DNA and reduce

physiological functions that can increase the growth of breast cancer cells. Lycopene is a compound that can counteract these free radicals. It is also able to slow down or even prevent the oxidation process of other molecules and eliminate free radicals in the body that can cause cell damage. Through these two mechanisms, suppressing cell multiplication and eliminating free radicals, lycopene can be used as a breast cancer therapy. [4].

A publication by Rao and Agarwai stated that lycopene compounds can reduce the risk of cancer by being a strong antioxidant. Lycopene has activity by suppressing damage to abnormal cells such as breast cancer cells. Lycopene can also increase cell interactions in increasing the hormone metabolites of cancer cells, thereby reducing the risk of breast cancer. This shows that lycopene compounds can be used as anti-cancer [5].

The cytotoxic activity of pummelo citrus fruit extract could be observed by testing it with MCF-7 cells through IC50 parameter. MCF-7 cells are a widely cultured type of breast cancer. Pummelo fruit extract which provides anticancer potential in MCF-7 cells it is assumed that the extract has anti-cancer potential. IC50 is a 50% concentration of the extract to kill breast cancer cells. Pummelo fruit extract containing lycopene can be assumed to have anticancer potential in breast cancer cells if it has an IC50 value of less than 1000 $\mu\text{g/mL}$ [6]

Background (Optional)

Based on the problems stated above, this study focused on the following problems:

1. Does pummelo fruit extract have cytotoxic activity against breast cancer cells?
2. What is the concentration of pummelo fruit extract that can inhibit and kill breast cancer cells?

Objective

This study was conducted with the aim of:

1. To determine the anti-cancer potential of pummelo fruit extract on breast cancer cells
2. To find out the concentration of pummelo fruit extract which can inhibit and kill breast cancer cells.

Hypothesis

The hypothesis in this study was that pummelo citrus fruit extract had cytotoxic activity on breast cancer cells

METHODS

Preparing Pummelo Citrus Fruit Extract

The plant material used was pummelo citrus fruit (*Citrus maxima*) from Sukomoro District, Magetan Regency, East Java. The pummelo fruit was extracted using the maceration method with 96% ethanol solvent. The dry extract of 300 grams of pummelo orange fruit *Simplicia* powder was macerated using 3 liters of 96% ethanol as a solvent.

Pummelo Citrus Cytotoxic Test

The anticancer potential of pummelo citrus fruit extract was conducted using the MTT method. The process of implementing the anticancer potential test by inserting several test cells as much as 1×10^4 cells/well in complete culture media consisting of FBS as the main cell nutrient, penicillin-streptomycin as a bacterial contaminant inhibitor, amphotericin-B as a fungal contaminant inhibitor, and RPMI 1640 as a carrier medium (volume of each well 100 μl) into 96-well plates and incubated in a 5% CO_2 incubator overnight. Furthermore, the test sample was given with a series of levels and made replications three times (triple), then incubated again overnight. On the third day, we added MTT reagent and after 4 hours the formazan crystals were formed in the living cells. Furthermore, a SDS stopper was added to stop the MTT reaction. Then, we performed the absorbance reading using an ELISA reader at a wavelength of 595 nm.

Data analysis

The data obtained will be used to calculate the IC50 value, where the percent viability had been calculated. Based on the absorbance value, we determined the percentage of cell viability with the following formula:

$$\% \text{ viability} = \frac{(\text{Treatment absorbance} - \text{media control absorbance})}{(\text{cell control absorbance} - \text{media control absorbance})} \times 100\%$$

After that, the IC50 value was calculated using the linear regression value.

RESULTS

Pummelo Citrus Fruit Extraction (*Citrus Maxima*)

The pummelo fruit was weighed as much as 300 grams then the extraction process was performed by the maceration method using 98% ethanol as a solvent. The viscous extract obtained was 11.5 grams. In this extraction process, the yield was 3.8%.

The Potential of Pummelo Oranges as an Anti-Breast Cancer Agent

Anti-breast cancer potential in pummelo citrus fruit extract by testing on MCF-7 cells. The determination of cytotoxic activity began with the value of the extract's absorbance curve with the percentage of living cells. The regression results on this curve were used to calculate the IC50 value. The IC50 value is a parameter of an extract capable of killing 50% of breast cancer cells. The results of the regression curve between the absorbance value of the extract and the percentage of living MCF-7 cells are presented in Table 1 and Figure 1 below.

Table 1. Levels of Pummelo Citrus Extract on the Living Breast Cancer Cells

Concentration ($\mu\text{g/mL}$)	% living cells
500	57.64023211
125	47.58220503
62.5	45.06769826
31.25	43.52030948
7.8	43.03675048

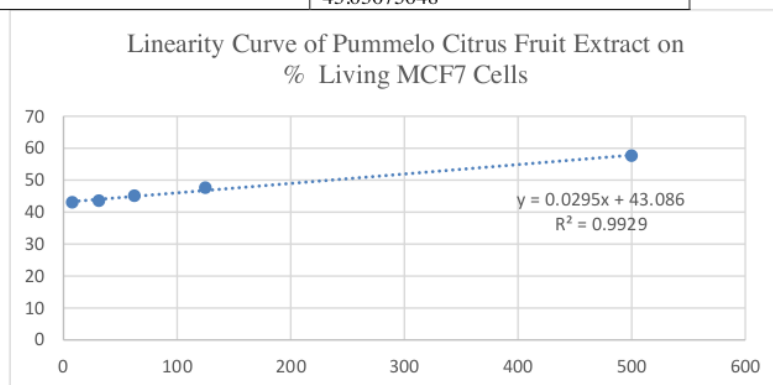


Figure 1: Linearity Curve of Pummelo Orange Extract on the Potential of Living Breast Cancer Cells

The results of the linearity curve of pummelo extract levels on the activity of living breast cancer cells showed the regression value was $y = 0.00295x + 43.086$, with a value of $r = 0.9964$. The value of $r = 0.9964$ indicated that there was a linear relationship between pummelo extract and the potential for living breast cancer cells. This showed that the regression value can be used to calculate the IC50 value. The IC50 value of pummelo orange extract against the potential of living cancer cells was 234 ($\mu\text{g/mL}$).

DISCUSSION

Pummelo Citrus Fruit Extraction (*Citrus Maxima*)

Pummelo citrus fruit was dry weighed as much as 300 grams then extracted by maceration method and used ethanol solvent 98%. The yield of viscous extract was 11.5 grams. In this extraction process, the yield was 3.8%. The yield was quite small because the water content in the pummelo orange was quite high so that the weight loss after drying using the oven was quite plenty. The yield can be used as a reference for the number of *Simplicia* that must be prepared for the extract needs to be used in research. The extraction of pummelo fruit used the maceration method. This was because this method had the advantages of being simple, easy to use, and can extract all active substances, both those that can withstand heating and those that were not. Ethanol 96% was chosen as a solvent because it was semi-polar so that it can optimally absorb lycopene. Ethanol 96% was chosen as the extraction solvent because the high concentration can extract the active substance maximally.

4.1.2 Potential of Pummelo Citrus as an Anti-Breast Cancer Agent

The anti-breast cancer potential of the fruit extract against these cells was to test it on MCF7 cells. MCF-7 cells are the most widely cultured cell type for breast cancer research. These cells were taken from the breast tissue cells of a 69-year-old Caucasian woman with blood type O with Rh-positive so that if the pummelo fruit extract had cytotoxic activity against MCF-7 cells, it had the potential to be anti-breast cancer. The determination of cytotoxic activity was using the absorption curve value of the extract with the percentage of living cells. The regression results on this curve were used to determine the IC50 value. The IC50 value is the value of an extract capable of killing 50% of breast cancer cells. The anti-cancer activity was seen using IC50 parameters. The smaller the IC50 value the greater the cytotoxic activity.

In the linearity curve of pummelo extract levels to the potential of live breast cancer cells, the regression value was $y = 0.00295x + 43.086$, with a value of $r = 0.9964$. The value of $r = 0.9964$ indicated that there was a linear relationship between pummelo extract and the potential for living breast cancer cells. This showed that the regression value can be used to calculate the IC50 value

The IC50 value of pummelo extract against the potency of living cancer cells was 234 ($\mu\text{g}/\text{mL}$). An extract is supposed to have anti-cancer activity if it has an IC50 value below 1000. This value indicated that the extract of pummelo orange had the potential as an anti-breast cancer. IC50 value 234 $\mu\text{g}/\text{mL}$ showed potential as a breast cancer agent although its potential was not strong. The potential of an extract is strong as an anti-cancer agent if the IC value of 50 is below 30 $\mu\text{g}/\text{mL}$. The pummelo extract has the potential as an anti-breast cancer agent due to the presence of lycopene compounds. Lycopene has potential as an anti-cancer by suppressing cell proliferation or multiplication mechanisms. In breast cancer patients, cell multiplication occurs very rapidly. Lycopene can also prevent oxidation of other molecules, minimizing free radicals so that cell damage does not occur. Free radicals are able to interact with DNA so that they can damage their physiological functions. Furthermore, lycopene is a compound that can minimize free radicals very well. Through these two mechanisms, namely suppressing cell multiplication and eliminating free radicals, lycopene can be used as a breast cancer therapy.

Lycopene is a selective compound, which only eliminates breast cancer cells without damaging normal cells. This is one reason for the use of lycopene in the pummelo citrus extract as an anti-breast cancer agent. The use of pummelo orange, which is a typical plant of Magetan Regency, can provide added value. The added value of pummelo orange as a superior commodity in the Magetan Regency is not only sweet to taste, high vitamin content but also as therapy or prevention against breast cancer cells.

CONCLUSION

In this study it can be concluded:

1. Pummelo fruit extract has potential as an anti-breast cancer
 2. IC50 value pummelo fruit extract 234 $\mu\text{g}/\mu\text{L}$, where at this level 50% of breast cancer cells die.
-

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