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RESEARCH ARTICLE

Potential of Pomelo Fruit (Citrus maxima) Extract on T47D, WiDr, and HepG2 Cancer Cells

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Abstract

Currently, alternative therapies are needed for breast, colon, and liver cancer patients. Those therapies are therapies that can selectively kill cancer cells without damaging other cells that have high development, such as hair and nails. One of those alternative therapies is the use of plants in cancer therapy. Pomelo has the potential as an anti-cancer agent because it contains phytochemical compounds, one of which is lycopene. Lycopene is a free anti-radical compound found in pomelo (citrus maxima). The lycopene content in pomelo fruit is more than in other parts, such as in 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 cervical cancer patients, cell multiplication occurs quite rapidly, so that with the presence of lycopene, cervical cancer cell growth can 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 cervical cancer cell growth. Lycopene is a compound that can counteract these free radicals and 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, namely suppressing cell multiplication and eliminating free radicals, lycopene can be used as a cancer therapy. The cytotoxic activity test of pomelo fruit extract was carried out using the MTT method on T47D, WiDr, and HepG2 cells. The pomelo fruit was extracted by maceration method using 96% ethanol solvent. Cancer cell cultures were transferred as many as $1x10^4$ cells/well in culture media consisting of FBS, penicillin-sterptomycin, amphoterizin-B, and RPMI 1640 into 96-well plates and incubated in a 5% CO_2 incubator overnight. Furthermore, the test samples were given a series of levels and replications were made three times (triplo), and then they were incubated again overnight. On the third day of testing, MTT reagent was added, and after 4 hours, formazan crystals in living cells would be formed. Furthermore, SDS stoper was added to stop the MTT reaction. Then, the absorbance reading was carried out using an elisa reader at a wavelength of 595nm and the determination of the IC50 value was then carried out as well. The results showed that the IC50 value of pomelo extract in T47D 50 cells was 954.34 µg/mL, in WiDr cancer cells was 130.70 µg/mL, and in HepG2 cancer cells were 1,660.257 µg / mL. This shows that pomelo extract has the potential as an anti-cancer agent for breast and colon cancer, but not for liver cancer. This research could contribute to the development of breast and colon cancer drugs.

Keywords: *Cytotoxic activity, Lycopene, T47D cell, WiDr cell, HepG2 cell.*

Introduction

Cancer is a type of disease that is most feared today. The high prevalence and the low cure rate are one of the reasons why this disease is such a scary thing. Based on data from the Ministry of Health, the incidence of cancer continues to increase. In 2018, 1.79 per 1000 residents developed cancer, whereas in 2013, it was still 1.4 per 1000 residents. Types of cancer that often cause death are breast cancer, colon cancer, and liver cancer. Colon cancer and liver cancer are types of cancer that rarely occur but often cause death, while breast cancer is a type of cancer that occurs in many women and often causes death. This is of particular concern, especially in efforts to treat the disease. Currently, chemotherapy is still an option for cancer treatment [1, 2]. Chemotherapy is a series of therapies commonly performed on breast, colon, and liver cancer patients. Some of the drugs used in cancer chemotherapy are fluorouracil, doxorubicin, and cylofosfamide.

Combination therapy is usually chosen to speed up the killing of cancer cells.

The mechanism of this drug occurs in the metastatic phase of all cells, so that it quickly kills all cells, including normal cells [3].Currently, alternative therapies are needed for breast, colon, and liver cancer patients. Those therapies are therapies that can selectively kill cancer cells without damaging other cells that have high development, including hair and nails. One alternative therapy is the use of plants in cancer therapy.

A plant that can be used as cancer therapy is pomelo (citrus maxima), which is a typical plant in Magetan district. Pomelo, which is abundant in Magetan district, needs to be developed as a potential anti-cancer agent. Pomelo has the potential as an anti-cancer agent because it contains phytochemical compounds, one of which is lycopene [4].

Lycopene is a free anti-radical compound found in pomelo fruit (citrus maxima). The lycopene content in pomelo 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 phases of cancer. In cervical cancer patients, cell multiplication occurs quite rapidly, so that with the presence of lycopene, cervical cancer cell growth can 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 cervical cancer cell growth. Lycopene is a compound that can counteract these free radicals and 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, namely suppressing cell multiplication and eliminating free radicals, lycopene can be used as a cancer therapy [4].The publication delivered by Rao and Agarwai stated that lycopene compounds could reduce cancer risk by being a powerful antioxidant. Lycopene had activity by suppressing damage to abnormal cells, such as cervical cancer cells. Lycopene could also increase cell interaction in increasing the hormone metabolites of cancer cells, so that it could reduce the risk of cervical cancer.

This showed that lycopene compounds could be used as anti-cancer [5].The cytotoxic activity of pomelo fruit extract can be seen by testing it with T47D, WiDr, and HepG2 cells through the IC50 parameter. T47D cells are a widely cultured type of breast cancer cell. WiDr cells are a type of colon cancer cell that has been widely developed. HepG2 cells are a type of liver cancer cell.

If the extract of pomelo fruit provides anti-cancer potential in T47D, WiDr, and HepG2 cells, then it can be said that the extract has anti-cancer potential. IC50 is a 50% concentration of the extract to kill cancer cells. Pomelo fruit extract containing lycopene can be said to have anticancer potential in breast, colon, and liver cancer cells if it has an IC50 value of less than 1,000 µg / mL [6].

Method

The Making of Pomelo Fruit Extract

The plant material used was pomelo fruit (citrus maxima) from Sukomoro District, Magetan Regency, East Java. The pomelo fruit that had been obtained was extracted using the maceration method with 96% ethanol solvent. The dry extract of 300 grams of pomelo fruit simplicia powder was macerated using 3 liters of 96% ethanol solvent.

Cytotoxic Test of Pomelo Fruit on T47D, WiDr, and HepG2 Cells

The anticancer potential of pomelo fruit extract was carried out using the MTT method. The process of implementing the anti-cancer potential test was by inserting a number of test cells as much as 1x104 cells/well in complete culture media consisting of FBS as the main cell nutrient, penicillin-sterptomycin as a contaminant preventive bacteria, amphoterizin-B as a fungal contaminant inhibitor, and RPMI 1640 as a carrier medium (volume of each well was 100 µl) into 96-well plates and being incubated in a 5% CO_2 incubator overnight.

Furthermore, the test samples were given with a series of levels and replications were made three times (triplo), and then they were

incubated again overnight. On the third day of testing with the addition of MTT reagent, after 4 hours, formazan crystals would be formed in living cells. Furthermore, SDS stoper was added to stop the MTT reaction. Then, the absorbance reading was carried out using an elisa reader at a wavelength of 595 nm.

Daya Analysis The data will be used to calculate the IC50 value, where the percentage of viability has been calculated. Based on the absorbance value, the data are used to determine the percentage of cell viability with the following formula:

$$\% \text{ of viability} = \frac{(abs\ treatment - abs\ media)}{(abs\ cell\ control - abs\ media)} \times 100\%$$

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

Results and Discussion

Pomelo Fruit (Citrus Maxima) Extract

The pomelo fruit was weighed as much as 300 grams and then extracted by maceration method using 98% ethanol solvent. The yield of thick 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 pommelo was quite high, so that the weight loss after drying using the oven was quite a lot. The yield could be used as a reference for the number of simplicia that must be prepared for the extract needed to be used in research.

The pomelo fruit extraction used maceration method. This was because this method had the advantages of being simple, easy to use, and able to extract all active substances that were resistant to heating and those that were not resistant to heating. 96% ethanol was chosen as a solvent because it was semi-polar, so that it could optimally absorb lycopene. 96% ethanol was chosen as the extraction solvent because of its high concentration which could filter out active substances maximally.

Potential of Pomelo Fruit as Anti-breast Cancer Agent

The anti-cancer potential of pomelo fruit extract against breast cancer cells is tested on T47D cells. T47D cells are cells that are taken from the ductal tumor tissue of a woman's breast, so if the pomelo fruit extract has cytotoxic activity against T47D cells, then it has the potential to be anti-breast cancer. Determination of cytotoxic activity began with the absorbance curve value of the extract with the percentage of living cells.

The regression results on this curve would then be used to determine the IC50 value. The IC50 value is the value of an extract capable of killing 50% of cancer cells. Anti-cancer activity was seen using IC50 parameter. The smaller the IC50 value is, the greater the cytotoxic activity will be. The results of the regression curve between the absorbance value of the extract and the percentage of living T47D cells are presented in Table 1 and Figure 1 below.

Table 1: Levels of pomelo fruit extract on the potential of living T47D cancer cells

Concentration (µg/mL)	% of Living Cells
500	78.70257038
62.5	108.7347463
31.25	105.0096339
15.625	105.716
7.8	114.7719974

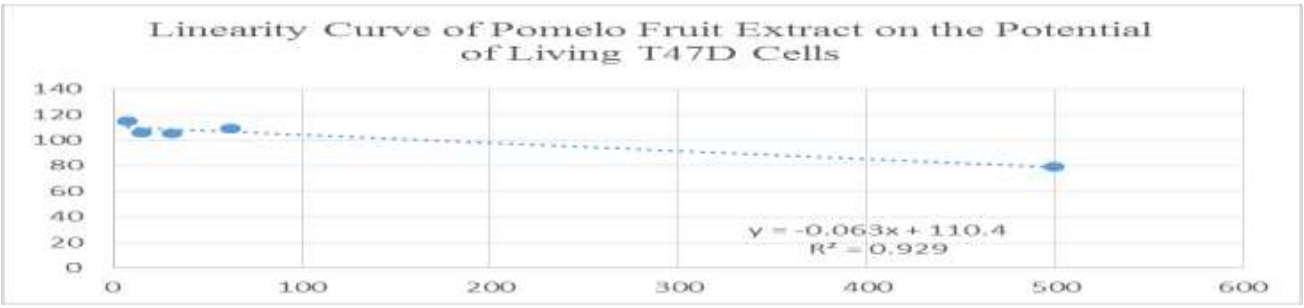

Figure 1: Linearity Curve of Pomelo Fruit Extract on the Potential of Living T47D Cells

In the linearity curve of pomelo fruit extract on the potential of living T47D cells, the regression value was y = -0.0633x + 110.41; with a value of r = 0.964. The value of r = 0.964 indicated that there was a linear relationship between the pomelo fruit extract and the potential of living T47D cells. This shows that the regression value can be used to determine the IC50 value. The IC50 value of the pomelo fruit extract on the potential of living cancer cells was 954.34 (µg/mL). An extract can be said to have anti-cancer activity if it has an IC value of below 1000 µg/mL.

This value indicates that pomelo fruit extract has the potential as an anti-breast cancer. IC50 value of 954.34 µg/mL indicates its potential as an agent for cervical cancer even though its potential is weak. The potential of an extract is said to be strong as an anti-cancer agent if the IC50 value is below 30 µg/mL.

The pomelo fruit extract has the potential as an anti-breast cancer agent due to the presence of lycopene compounds. Lycopene has the potential as an anti-cancer by suppressing cell proliferation or multiplication mechanisms. 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 fat and 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, 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 pomelo fruit extract as an anti-breast cancer agent [7, 8].

Potential of Pomelo Fruit as Anti-colon Cancer Agent

The anti-cancer potential of pomelo fruit extract against colon cancer cells is tested on WiDr cells. WiDr cells are cells taken from human colon cancer cells that have been isolated, so if the pomelo fruit extract has cytotoxic activity against WiDr cells, then it means that it has the potential to be anti-colon cancer. Determination of cytotoxic activity began with the absorbance curve value of the extract with the percentage of living cells.

The regression results on this curve would then be used to determine the IC50 value. Anti-colon cancer activity was assessed using the IC50 parameter. The smaller the IC50 value is, the greater the cytotoxic activity will be. The results of the regression curve between the absorbance value of the extract and the percentage of living WiDr cells are presented in Table 2 and Figure 2 below.

Table 2: Levels of pomelo fruit extract on the potential of living WiDr cancer cells

Concentration (µg/mL)	% of Living Cells
500	9.402795426
125	55.86734694
62.5	58.03571429
31.25	57.5255102
15.625	61.81972789
7.8	61.81972789

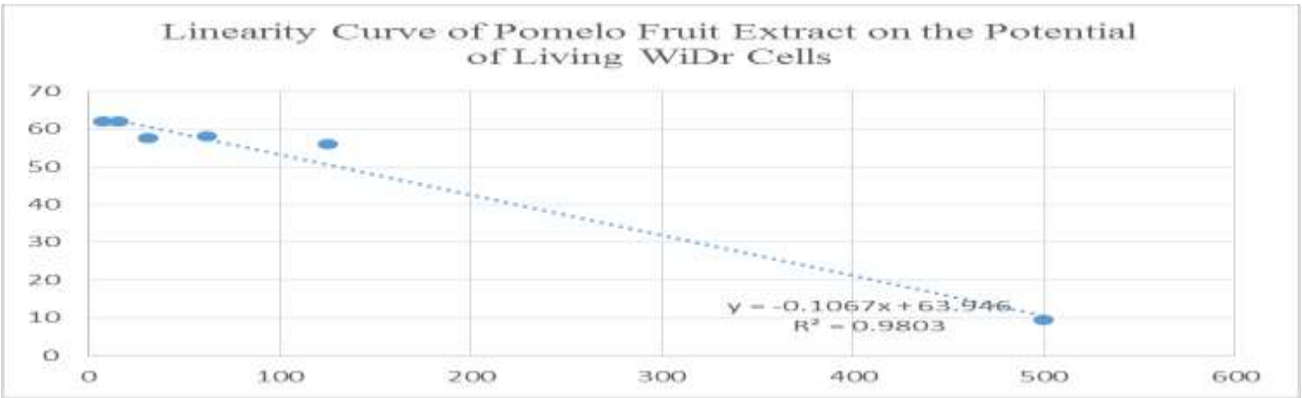

Figure 2: Linearity curve of pomelo fruit extract on the potential of living WiDr cells

In the linearity curve of pomelo fruit extract on the potential of living WiDr cells, the regression value was y = -0.1067x + 63.946; with a value of r = 0.990. The value of r = 0.990 indicated that there was a linear relationship between the pomelo fruit extract and the potential of living WiDr cells. This shows that the regression value can be used to determine the IC50 value. The IC50 value of the pomelo fruit extract on the potential of live cancer cells was 130.70 (µg/mL).

An extract can be said to have anti-cancer activity if it has an IC50 value of below 1000 µg/mL. This value indicates that pomelo fruit extract has potential as an anti-colon cancer agent. The IC50 value of 130.70 µg/mL indicates its potential as a medium anti-colon cancer agent. The extract of pomelo fruit has the potential as an anti-colon cancer agent due to the presence of lycopene compounds. Lycopene has the potential as an anti-cancer with dual mechanisms, namely suppressing cell proliferation or multiplication.

In colon 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 fat and will damage their physiological functions, which in turn can lead to the development of colon cancer. Lycopene is a very effective free radical eliminator. Through these two mechanisms, namely suppressing cell multiplication and eliminating free radicals, lycopene can be used as a colon cancer therapy. Lycopene is a selective compound, which only eliminates colon cancer cells without damaging normal cells [9, 10].

Potential of Pomelo Fruit as Anti-liver Cancer Agent

The anti-cancer potential of pomelo fruit extract against liver cancer cells is tested on HepG2 cells. HepG2 cells are cells taken from the liver tissue of a person suffering from hepatocellular carcinoma, so if the pummelo fruit extract has cytotoxic activity against HepG2 cells, then it means that it has the potential to be anti-liver cancer. Determination of cytotoxic activity began with the absorbance curve value of the extract with the percentage of living cells.

The regression results on this curve would then be used to determine the IC50 value. Anti-liver cancer activity was seen using the IC50 parameter. The results of the regression curve between the absorbance value of the extract and the percentage of living WiDr cells are presented in Table 3 and Figure 3 below.

Table 3: Levels of pomelo fruit extract on the potential of living HepG2 cancer cells

Concentration (µg/mL)	% of Living Cells
500	114.2490372
250	125.9503386
125	134.9435666
62.5	136.1444695
31.25	140.5502201

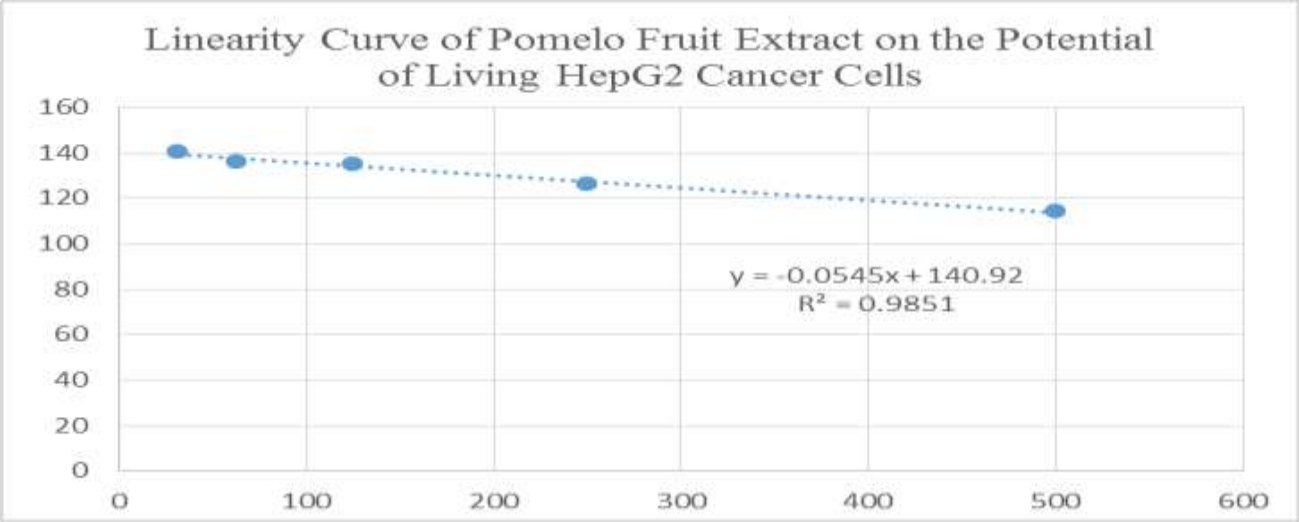

Figure 3: Linearity curve of pomelo fruit extract on the potential of living HepG2 cancer cells

In the linearity curve of pomelo fruit extract on the potential of living HepG2 cells, the regression value was y = -0.0545x + 140.92; with a value of r = 0.992. The value of r = 0.992 indicated that there was a linear relationship between the pomelo fruit extract and the potential of living HepG2 cells. This shows that the regression value can be used to determine the IC50 value. The IC50 value of pomelo fruit extract against the potential of living cancer cells was 1660.257 (µg/mL).

An extract can be said to have anti-cancer activity if it has an IC50 value of below 1000 µg/mL. This shows that pomelo fruit extract does not have cytotoxic activity against liver cancer cells.

The cytotoxic activity test of pomelo fruit extract on three types of cancer cells showed that this extract had the most potential against colon cancer cells compared to breast cancer or liver cancer cells. This occurred due to differences in the characteristics of these cancer cells. Differences in cell characteristics will cause the results of the mechanism of lycopene to these cells to be also different [11, 12, 13].

Conclusion

- Pomelo fruit extract has cytotoxic activity on T47D cancer cells with an IC50 value of 954.34 µg/mL
- Pomelo fruit extract has cytotoxic activity on WiDr cancer cells with an IC50 value of 130.70 µg/mL
- Pomelo fruit extract does not have cytotoxic activity on HepG2 cancer cells with an IC50 value of 1,660.257 µg/mL

References

1. Departemen Kesehatan (2019) Hari anti kanker Sedunia 2019. Kementrian Kesehatan. Jakarta
2. Ramli M (2015) Update Breast Cancer Management Diagnostic and Treatment. Majalah Kedokteran Andalas., 38 (1): 28-53.
3. Agustini D, Emma S, Abdullah R (2015) Kualitas Hidup Pasien Kanker Servik Dengan Terapi Kombinasi Flourourasil, Doxorubicin, Dan Cyclofosfamide. Jurnal Farmasi Klinik Indonesia, 4(3): 175-185.
4. Navitri A, dan Monica M (2012) Uji Aktivitas Antiradikal Bebas Ekstrak Buah Jeruk Bali (Citrus Maxima Burm Fz) Dengan Metode DPPH (1, 1 Diphenyl-2-Pikrylhidrazyl). Journal of Chemistry, 1(2): 1-6.
5. Rao A, Agarwai S (2013) Role of Antioxidant Lycopene in Cancer and Heart Disease. Journal of the American Collage of Nutrition, 19.
6. Amir H, dan Murcitro B (2017) Uji Mikrotetrazolium (MTT) Ekstrak Metanol Daun Phaleria Macrocarpa (Scheef) Boerl Terhadap Sel Kanker Servik MCF-7. Jurnal Pendidikan dan Ilmu Kimia, 1(1): 27-32.
7. Bohm F, Tinkler JH, Truscott TG (1995) Carotenoids protect against cell membrane damage by the nitrogen dioxide radical. Nature Med., 1: 98-99.
8. Boileau TWM, A C Moore, J W Erdman (1999) Carotenoids and Vitamin A. In: Antioxidant Status, Diet, Nutrition, and Health (Papas, A. M., ed.), 133-158. CRC Press LLC, Boca Raton, FL.
9. Birt D F, S Hendrich, W Wang (2001) Dietary agents in cancer prevention: flavonoids and isoflavonoids. Pharmacol. Ther., 90: 157-177.
10. Breinholt V, S T Lauridsen, B Daneshvar, J Jakobsen (2000) Dose-response effects of lycopene on selected drug-metabolizing and antioxidant enzymes in the rat. Cancer Lett., 154: 201-210.
11. Sutejo, Putri Meiyanto (2016) Selektivitas Ekstrak Etanolik Buah Makasar (Brucea Javanica) pada Kanker Payudara Metastatis Secara In Vitro. Journal of Agromedicine and Medical Science, 1-5.
12. Nurani (2012) Uji Sitotoksisitas dan Antiproliferatif Sel Kanker Payudara T47D Dan Sel Vero Biji Nigella sativa, L. Jurnal Ilmiah Kefarmasian, 2(1): 17-29.
13. Sutejo, Putri Meiyanto (2016) Ekstrak Etanolik Awar-Awar (Ficus Septica) Sebagai Agen Kemopreventif Selektif Pada Berbagai Macam Sel Kanker. Nurseline Journal, 190-197.

