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Cytotoxic Potential of *Mangifera indica* L var Arumanis Rind Extract and Fraction Against T47D Cells with Microtetrazolium (MTT) Method

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Abstract−Breast cancer is a major problem in the health sector, one type of malignant cancer in Indonesia and the world. Medical therapy for cancer patients has had side effects such as nausea, vomiting, weakness, diarrhea and hair loss. The arumanis mango rind, in previous research, contains secondary metabolites of flavonoids, phenolics, saponins and tannins. Arumanis mango rind extract has been studied to have antioxidant activity with an IC_{50} of 18.29 µg/ml and a toxic, toxicity test with an LC_{50} of 169.04 µg /ml. The aim of the research was to determine the cytotoxic potential of arumanis mango rind extract and fractions against T47D cancer cells using the Microtetrazolium (MTT) method from the percentage of cancer cell viability, IC_{50}, and cancer cell morphology. It was using ethanol extract, n-hexane fraction, ethyl acetate fraction, and n-butanol fraction, with concentrations of 1000, 100, 10, 1, 0.1 µg/ml against T47D cancer cells. Data were the life cell absorbance obtained from the ELISA reader, to calculate the percentage of T47D cell viability, obtain IC_{50}, then observe the morphology of the cancer cells. The results of the percentages of cell viability (µg/ml) from the ethanol extract were 81.1: 103.5; 137.3; 163; 165.5, n-hexane fraction were 28.5; 82.7; 105.1; 130.5; 132.5, ethyl acetate fraction were 15.9; 27.2; 101.7; 134.1; 162.7, n-butanol fraction were 32.6; 75.6; 82.8; 122.3; 146.4, doxorubicin as a comparison were 22.6; 38.4; 45.4; 53.4; 56. The IC_{50} results of the ethanol extract, the n-hexane fraction, the ethyl acetate fraction, the n-butanol fraction, and doxoburicin were 32.21; 616.59; 90.57; 338.84, and 1.44. The best of cancer cell morphology was doxorubicin, ethanol extract, and ethyl acetate fraction. The conclusion that the extract and fraction of arumanis mango rind had cytotoxic potential against T47D cancer cells from the percentages of cell viability, IC_{50} and cancer cell morphology, and the best of result are ethanol extract, ethyl acetat fraction, also doxorubicin as comparison drug.

Keywords: Arumanis; Mangifera indica; MTT; Rind; T47D cells.

1. INTRODUCTION

Cancer is an abnormal growth of body tissue cells that turns malignant.(Curigliano et al., 2023) Cancer is a disease that still cannot be completely cured.(Yudistira, 2017) Breast cancer ranks fifth in cancer deaths overall, and meanwhile, is the most common cause of death in women.(Wilkinson & Gathani, 2022) Breast cancer is a disturbance in the growth of normal mammary cells where abnormal cells arising from normal cells multiply and infiltrate the lymphatic tissue and blood vessels.(Bannour et al., 2018)

One in six deaths in the world occurs due to cancer and is the second cause of the highest number of deaths in the world with a death toll of around 9.6 million people. The incidence of cancer in Indonesia in 2013-2018 was 14 per 1000, an increase of 28.6% to 1.8 per 1000 population. Breast cancer ranks first with a prevalence of 40 per 100,000, an increase from 2002 with a figure of 26 per 1000. The type of cancer most commonly found in patients in Indonesia is breast cancer at 28.7%, then cervical cancer at 12.8%.(Musthika et al., 2018).

Breast cancer patients can be treated with surgery, chemotherapy and radiation, but due to the Multidrug Resistance (MDR) mechanism, the effectiveness of chemotherapy drugs is reduced, sometimes this medical therapy results in nausea, vomiting, weakness, diarrhea and hair loss.(Budny et al., 2019) Due to the many side effects, it is very necessary to test the potential of natural ingredients as chemopreventive agents which have the potential to be chemotherapy companion agents, which in this case are cheaper, easier to obtain, and have minimum side effects.(Kashyap et al., 2022)

One plant that can be used as traditional medicine is the arumanis mango plant. The results of previous research show that arumanis mango peel as an antioxidant in the form of extract and infusion respectively has an Inhibitory Concentration (IC_{50}) of 12.46 µg/ml and 46.92 µg/ml in the very strong category. (Taswin & Toyibah, 2020).This research is based on the results of our research in 2023 that the antioxidant activity of the ethanol extract of arumanis mango peel (KBMA) IC_{50} is 18.29 µg/ml (very strong), and the toxicity LC_{50} is 169.04 µg/ml (toxic), accompanied by positive phytochemical screening for flavonoids, phenols, terpenoids, steroids, saponins and tannins.(Ayyun et al., 2023)

Arumanis mango skin contains secondary metabolites of flavonoids, phenolics, terpenoids, steroids, saponins, and tannins, where flavonoids have antioxidant activity. (Maldonado-Celis et al., 2019) The ethanol extract of arumanis mango rind contains many other secondary metabolite compounds, so it must be fractionated. The antioxidant properties of KBMA extract as a very strong antidote to free radicals, research has been directed towards the cytotoxic potential of KBMA extract as an anti-breast cancer agent, because no one has researched it. In this research, it was taken from ethanol extract and fractionated KBMA extract.(Abdullah et al., 2015) Previous research on pineapple and ethanol extracts of honey mango, golek and arumanis seeds observed their anti-breast cancer effect on T47D cancer cells. Research on the cytotoxic potential of anti-breast cancer of ethanol extract and fractionation of KBMA extract has never been carried out using T47D cancer cells using the MTT method. The mango plant also contains the compound mangiferin which has been

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studied as an anti-breast cancer agent. Mangiferin is a xanthon compound resulting from purification from polar solvents such as ethanol, not semi-polar or non-polar solvents.(Yap et al., 2021)

Testing for breast cancer potential in this study was carried out on ethanol extract and n-hexane, ethyl acetate and n-butanol fractions, using T47D breast cancer cells. T47D cells are cells that are very sensitive to doxorubicin which can express p-53 protein mutations, are easy to handle, have unlimited replication capability, have high homogenity, and can be replaced with frozen stock if contamination occurs.(Safitri et al., 2021)

The method used is Microtetrazolium or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT assay) which forms a purple formazan compound. This method uses the Enzyme-Linked Immunosorbent Assay (ELISA reader) to observe the absorbance of compounds. This MTT method has the advantage that the test is more sensitive, faster, and can measure many samples at one time[(Kumar et al., 2018) The aim of the research was to determine the cytotoxic potential of arumanis mango rind extract and fractions against T47D cancer cells using the Microtetrazolium (MTT) method from the percentage of cancer cell viability, IC_{50}, and cancer cell morphology.

2. RESEARCH METHODS

2.1. The Extraction using the Maceration Method

Fresh arumanis mango rind (KBMA) was weighed 2 kg, cleaned, washed, drained, chopped into small pieces, macerated with 96% ethanol (1:10), for 3 x 24 hours. Perform repeated maceration until the solvent is clear, concentrate using a rotary evaporator to become a thick KBMA ethanol extract.(Abubakar & Haque, 2020)

2.2. Fractionation

The thick ethanol extract was weighed, dissolved in 100 ml of distilled water in a beaker, inserted into a 250 ml separating funnel, added 100 ml of n-hexane solvent. After that, shake it, let it sit for ± 15 minutes, take the n-hexane layer (top), and the water layer (bottom) followed by ethyl acetate solvent. The n-hexane layer was concentrated using a rotary evaporator, resulting in a thick n-hexane KBMA fraction. The ethyl acetate layer was taken, the water layer was dissolved with n-butanol solvent, each fraction was concentrated with a rotary evaporator into thick ethyl acetate and n-butanol fractions.(Abubakar & Haque, 2020)

2.3. Testing of Non-Specific and Specific Parameters of KBMA Extracts and Fractions

Observations were made on the KBMA extract and fraction; organoleptic, yield percentage, drying loss percentage, and determination of total ash content.(Kemenkes RI, 2017)

2.4. Phytochemical Screening of Arumanis Mango (Mangifera indica L) Rind Extracts and Fractions

Phytochemical test with the addition of certain reagents to determine the secondary metabolite content in KBMA extracts and fractions; Alkaloid Test, Flavonoid Test, Phenol Test, Saponin Test, Steroid/terpenoid Test and Tannin Test.(Abubakar & Haque, 2020)

2.5. Test the cytotoxic (anti-breast cancer) potential of T47D cancer cells using the microtetrazolium (MTT) method [10]

2.5.1. Sterilization

Sterilization is carried out on glassware that has been washed and dried, wrapped in aluminum foil, heat-resistant equipment is sterilized in the oven, at 175^{o}C for 2 hours, and non-heat-resistant, in an autoclave, at 121oC for 15 minutes. Sterilization using Laminar Air Flow (LAF) is carried out by turning on the Ultraviolet (UV) lamp for 15 minutes before use. The work space should be sprayed with 70% alcohol (CCRC, 2014)

2.5.2. Cell Growth

The breast cancer cells used, namely T47D cells, were purchased from the Biomedical Laboratory collection of the Faculty of Medicine, Andalas University. Cancer cells removed from the freezer (-80°C) are warmed in a water bath, temperature 37°C for 2-3 minutes. After thawing, the cancer cells from the cryotube were transferred into a conical centrifuge tube containing 10 ml of RPMI medium (Roswell Park Memorial Institute). centrifuge 5 minutes. The result is that there are 2 layers, namely the supernatant (top part) is removed and the pellet (bottom part) of the cancer cells is taken and put into a flask, 10 ml of RPMI media is added, the flask is incubated in an incubator at 37°/ 5% CO2 for 3-4 hours, then viewed in under a microscope, are the cells attached to the flask layer, if the number of cells in the flask reaches 70-85% cofluent, then the cells are harvested.(Ryu et al., 2013)

2.5.3. Cancer Cell Counting

Discard the RPMI media in the flask, add 1 ml Phosphate Buffer Saline (PBS) 2 times, then discard the PBS, add 1 ml trypsin-EDTA, incubate 3-5 minutes, temperature 37°C/ 5% CO2, add 5 ml RPMI media to inactivate trypsin and carry out resuspension, observe with an interted microscope to find out cells that have separated from the flask. Cancer cells

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that are ready to be harvested, take 10 µl and pipet them into a hemacytometer, then count the number of cancer cells, and place them on the counter according to the calculation. Perform calculations under an interted microscope.(CCRC, 2014)

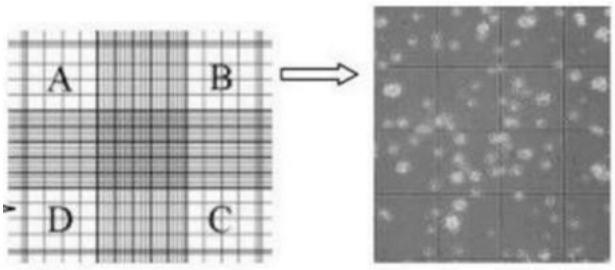

Figure 1. *Hemacytometer* (CCRC, 2014)

$$\text{Number of Cell suspensions} = \frac{\text{The Total number of cells required}}{\text{The number of cells is counted/ ml}} \quad (1)$$

2.5.4. Placing Cells on a 96-well Plate

Insert the cell suspension and RPMI media that have been made and calculated according to calculations into a 96-well plate, incubate for 24 hours, temperature 37°C/ 5% CO2, but leave the bottom 6 wells for cell control and media control.(CCRC, 2014)

2.5.5. Making and Placing the Sample Solution on the 96 Well Plate

The sample was weighed 10 mg in a container, dissolved in 10 ml DMSO, stirred with an ependroff tube. Next, the cells are taken from the incubator and the cell medium is discarded. A total of 100 µl of PBS was added into all wells containing cells and thrown back, adding 100 µl of sample solution with concentrations of 1000, 100, 10, 1, and 0.1 µg/ml, through dilution. Do the same thing with the fractions of n-hexane, ethyl acetate, n-butanol and doxorubicin (comparator). As controls, control cells and RPMI media control were used. This process was carried out 3 times and the plate was again incubated for 24 hours at 37oC/5% CO2.(CCRC, 2014)

2.5.6. Addition of MTT Solution

The cell media resulting from previous incubation was discarded, washed with PBS. Add 100 µl of MTT solution to the well, incubate the 96 well plate, 3-4 hours, temperature 37°C/5% CO2. After incubation, you can see formazan crystals with a color change to purple. Observe the cells with a microscope. Formazan crystals were dissolved in 100 µl DMSO and their absorbance was measured using the Enzym-Linked immunosorbent Assay (ELISA reader) at a wavelength of 550-600 nm. The MTT solution was stopped with 1% SDS in 0.01 N HCl.
Absorbance data from each well is converted to the cell viability percentage formula:

$$\text{Viability Percentage of T47D Cells} = \frac{A-B}{C-B} \text{ x } 100\% \quad (2)$$

Information:
A = absorbance of treatment (cells + culture medium + sample)
B = average absorbance of culture medium control
C = average absorbance of control cells (cells + culture medium)
The results of the cell viability percentage are entered into the graphpad prism 9 or excel application, the IC_{50} value is obtained, then observe the morphology of the cancer cells descriptively under a microscope. [10

2.6. Data Analysis

The cytotoxic potential of ethanol extract, KBMA fraction and Doxorubicin (comparator), in inhibiting or killing T47D breast cancer cells is known from the IC_{50} value. The use of absorbance data obtained from the ELISA reader is calculated using the cell viability percentage formula. The data was analyzed using the graphpad prism 9 or excel application, the IC_{50} value was obtained. Furthermore, the percentage of cancer cell viability for each concentration was analyzed using one-way ANOVA, followed by the Duncan and Tukey test, while the cancer cell morphology analysis was descriptive. (Safitri et al., 2021).

3. RESULT AND DISCUSSION

3.1 Collection and Extraction Results and Fractionation of Arumanis Mango Rind Waste (*Mangifera indica* L)

Arumanis mango rind waste (*Mangifera indica* L) was taken from the Pauh District, Padang City, from the fruit. The ethanol extract of arumanis mango rind (Mangifera indica L) obtained from the maceration process was 180.23 grams from 2 kg of fresh samples of arumanis mango rind. The characteristics of arumanis mango (Mangifera indica L) rind

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extract are; a. Organoleptic examination of arumanis mango rind extract, a) Form: thick extract, b) blackish brown color, c Odor: Typical, d) Taste; Bitter. The yield percentage obtained from the ethanol extract of arumanis mango peel is 9.01 %, The average drying loss obtained from the ethanol extract of arumanis mango peel is 4.30 %, The average ash content obtained obtained from 0.37% ethanol extract of arumanis mango rind. The weight of the n-hexane fraction obtained was 1.62 grams. Percentage yield of n-hexane fraction based on thick extract is 0.89% G. Weight of the ethyl acetate fraction obtained is 2.13 grams. Percentage yield of n-ethyl acetate fraction based on thick extract is 1.18% Weight of n- butanol obtained was 1.03 grams. Percentage yield of n-butanol fraction based on thick extract is 0.57%(Kemenkes RI, 2017)

Figure 2. Extract of Arumanis Mango Rind

Figure 3. Fractions of Arumanis Mango Rind

3.1.1 Result of T47D Cell Viability Percentage

The results of the percentages of cell viability (µg/ml) from the ethanol extract were 81.1: 103.5; 137.3; 163; 165.5, n-hexane fraction were 28.5; 82.7; 105.1; 130.5; 132.5, ethyl acetate fraction were 15.9; 27.2; 101.7; 134.1; 162.7, n-butanol fraction were 32.6; 75.6; 82.8; 122.3; 146.4, doxorubicin as a comparison were 22.6; 38.4; 45.4; 53.4; 56. (Zhang et al., 2020)

Table 1. Results of T47D Cell Viability Percentage

	Viability Percentage of T47D Cell (µg/ml)				
Group of Sample	1000	100	10	1	0.1
Ethanol Extract	81.1	103.5	137.3	163	165.5
Hexane Fraction	28.5	82.7	105.1	130.5	132.6
Ethyl Acetat Fraction	15.9	27.2	101.7	134.1	162.7
Buthanol Fraction	32.6	75.6	82.8	122.3	146.4
Doxorubicin inj	22.6	38.4	45.4	53.4	56

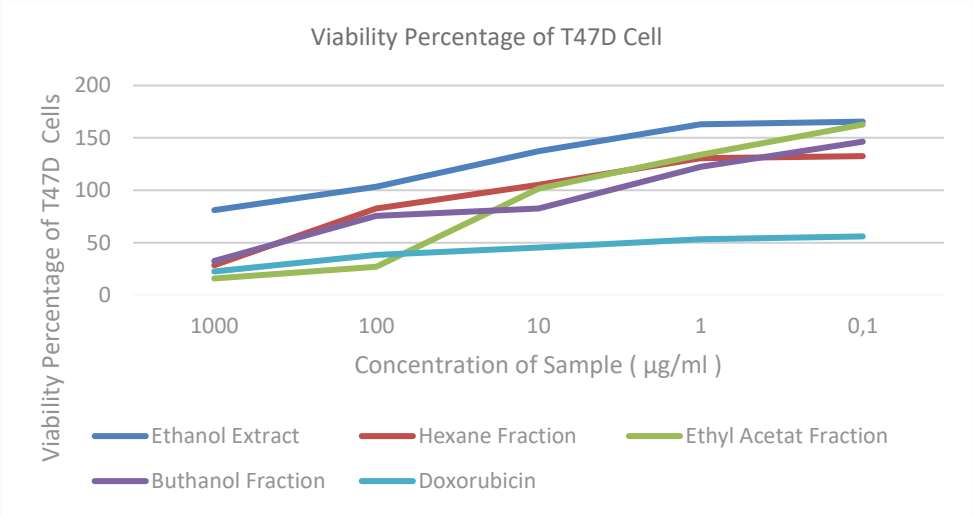

Figure 4. Graphic of T47D Cell Viability Percentage

3.1.2 Results of Inhibitory Concentration 50 (IC_{50})

The IC_{50} results of the ethanol extract were 32.21, which is toxic, the n-hexane fraction were 616.59, is toxic, the ethyl acetate fraction were 90.57, is toxic, the n-butanol fraction were 338.84, is toxic and doxorubicin were 1.44, is very toxic. The best cancer cell morphology was doxorubicin, ethanol extract, and ethyl acetate fraction. The conclusion that the extract and fraction of arumanis mango rind had cytotoxic potential against T47D cancer cells from the percentages of cell viability, IC_{50} and cancer cell morphology.(Kalliokoski et al., 2013)

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Table 2. Result of Inhibitory Concentration 50 (IC_{50}) value

Group of Sample	IC_{50} value
Ethanol Extract	32.21
Hexane Fraction	616.59
Ethyl Acetat Fraction	90.57
Buthanol Fraction	338.84
Doxorubicin	1.44

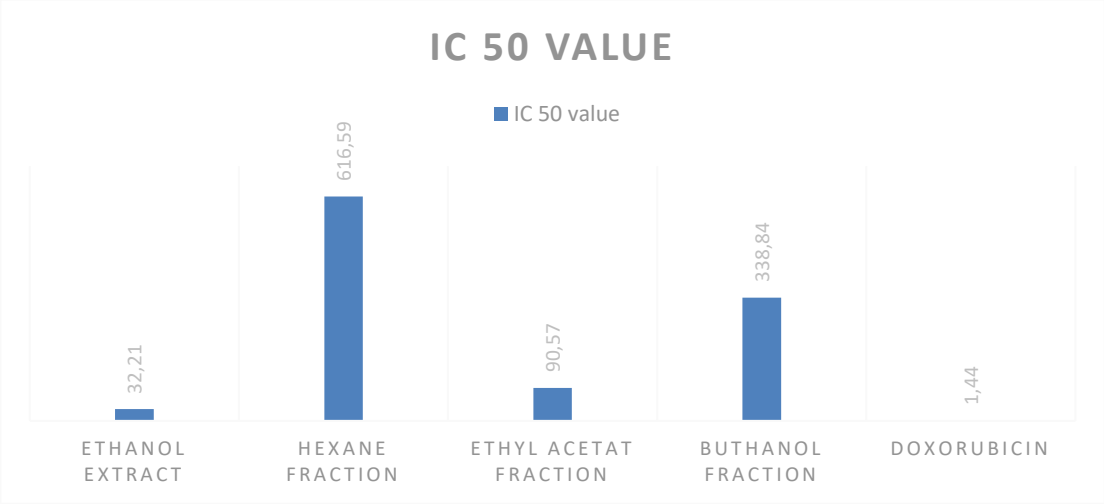

Figure 5. Graphic of IC_{50} value

3.1.3 Results of Morphology of T47D Cells

The analysis is seen from the cell morphology results that the higher the concentration, the greater the number of dead cells seen from the yellow or orange color produced by the MTT solution. Doxorubicin, Ethanol Extract, and Ethyl Acetat Fraction are the best description against the morphology of T47D cells.(Safitri et al., 2021)

3.2 Discussion

Cytotoxic activity testing was carried out with the aim of determining the ability of the ethanol extract of arumanis mango peel (KBMA) and its fractions to inhibit the growth of breast cancer cells. The cytotoxic activity test was carried out in vitro on T47D breast cancer cells using the Microtetrazolium (MTT) method. The research was carried out starting from making extracts, making n-hesane fractions, ethyl acetate fractions, and n-butanol fractions, cell culture, making and giving test solutions, giving MTT solutions, and reading absorbance using an ELISA reader at a wavelength of 570 nm. (Kumar et al., 2018). The extraction process uses the maceration method, namely an extraction method without heating. The fractionation process is simply carried out using a separating funnel, between distilled water and other solvents based on the level of polarity, then the hexane fraction, ethyl acetate fraction and n-butanol fraction are obtained.(Abubakar & Haque, 2020)

Next, the KBMA extract and fraction were intervened on T47D breast cancer cells. T47D breast cancer cells are a continuous cell line isolated from a woman's breast ductal tumor tissue. These cells are often used in in vitro cancer research, because these cells are easy to handle, have unlimited replication capacity, high homogeneity and can be frozen stock to make research easier. (Burdall, et al, 2003)

Microtetrazolium (MTT) is a cytotoxic method for determining the number of living cancer cells based on changing a yellow Microtetrazolium (MTT) solution into purple formazan crystals. The brighter the purple color indicates the higher the cell viability. In this research method, the purple color in the cell well indicates the formation of formazan crystals due to the reduction reaction of tetrazolium salt (MTT) in the respiratory chain in the mitochondria of living cells, while the yellow or orange color indicates that the cell is dead.(Kumar et al., 2018).

The meicrotetarzolium (MTT) method provides accurate test results because it provides a relationship between the number of active cells and the absorbance obtained from the measurements used to determine the IC_{50} value. The KBMA extract and fraction used in this stotoxic test have 5 variations, namely 1000 µg/ml; 100 µg/ml; 10 µg/ml; 1 µg/ml; 0.1 µg/ml, and the positive control was chosen Doxorubicin inj as a comparison because it is an effective anticancer drug and is often used as a chemotherapy agent in cancer.

The morphology of T47D breast cancer cells before treatment with the KBMA extract and fraction samples is as shown in the following picture below.(CCRC, 2014).

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Figure 6. T47D cell

The morphology of living cells has an oval shape that fits together at the bottom of the culture container. Meanwhile, dead cells are round, dark in color, scattered and floating. The cell morphology of KBMA extract and the n-hexane fraction, ethyl acetat fraction, and n-butanol fraction with 5 concentration variations looks as follows along with doxorubicin, also in 5 concentration variations.

1000 µg/ml 100 µg/ml 10 µg/ml 1 µg/ml 0,1 µg/ml

Figure 7. Morphology of T47D cells after being given ethanol extract of Arumanis Mango Rind in 5 variation concentrations

The analysis is seen from the cell morphology results that the higher the concentration, the greater the number of dead cells seen from the yellow or orange color, the more purple it means there are still many living cells produced by the MTT solution.

1000 µg/ml 100 µg/ml 10 µg/ml 1 µg/ml 0,1 µg/ml

Figure 8. Morphology of T47D cells after being given Hexane fraction of Arumanis Mango Rind in 5 variation concentrations

The analysis is seen from the cell morphology results that the higher the concentration, the greater the number of dead cells seen from the yellow or orange color, if the color is purple, there are still many living cells produced by the MTT solution.

1000 µg/ml 100 µg/ml 10 µg/ml 1 µg/ml 0,1 µg/ml

Figure 9. Morphology of T47D cells after being given Ethyl Acetat fraction of Arumanis Mango Rind in 5 variation concentrations

The analysis can be seen from the results of cell morphology, that the higher the concentration, the greater the number of dead cells seen from the color, if the more purple, the more living cells will be produced by the MTT solution.

1000 µg/ml 100 µg/ml 10 µg/ml 1 µg/ml 0,1 µg/ml

Figure 10. Morphology of T47D cells after being given Buthanol fraction of Arumanis Mango Rind in 5 variation concentrations

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The analysis is seen from the results of cell morphology that the higher the concentration, the greater the number of dead cells, seen from the purple color produced by the MTT solution.

1000 µg/ml 100 µg/ml 10 µg/ml 1 µg/ml 0,1 µg/ml

Figure 11. Morphology of T47D cells after being given Buthanol fraction of Arumanis Mango Rind

The analysis is seen from the cell morphology results that the higher the concentration, the greater the number of dead cells seen from the yellow or orange color produced by the MTT solution.

The anticancer ability of a compound to inhibit the growth of T47D breast cancer cells can be seen visually after administration of mcrotetrazolium (MTT) solution. Living cells will be marked by a color change from yellow to purple, which is caused by living cells absorbing tetrazolium salts and breaking them down into formazan crystals by the succinate tetrazolium reductase system. Meanwhile, dead cells will be characterized by not changing color or remaining yellow. Result of T47D cell morphology is the best of description in doxorubicin, ethanol extract and ethyl acetat fraction

In this study, it was discovered that the KBMA extract and fraction had an influence on the viability of T47D breast cancer cells, with a decrease in the percentage of cell viability found as the extract concentration increased.

The concentration of the KBMA extract and fraction has a relationship with the percentage of cell viability, namely determining a simple linear regression equation using a graph of concentration and cell viability, which can then be determined by the concentration of the extract and KBMA fraction which can inhibit cell viability by 50%.

Each sample starting from the KBMA ethanol extract and n-hexane fraction, ethyl acetate fraction, and n-butanol fraction intervened on T47D cells, producing absorbance from the ELISA reader with a wavelength of 570 nm. This process was carried out three times, and the results of the percentages of cell viability (µg/ml) from the ethanol extract were 81.1: 103.5; 137.3; 163; 165.5, n-hexane fraction were 28.5; 82.7; 105.1; 130.5; 132.5, ethyl acetate fraction were 15.9; 27.2; 101.7; 134.1; 162.7, n-butanol fraction were 32.6; 75.6; 82.8; 122.3; 146.4, doxorubicin as a comparison were 22.6; 38.4; 45.4; 53.4; 56.

Finally, it produces a graph in the form of a simple linear regression equation, where the regression equation is y = a + bx, where the values a and b are obtained from the slope of the graph. Then replace the y value to 50% so that the x value is obtained. To get a viability value of 50% (IC_{50}) the antilog value of x is used so that finally the IC_{50} is obtained from each sample, and the R2 value is 1.

The Inhibitory Concentration 50 (IC_{50}) is a measure of the potency of a substance in inhibiting a specific biological or biochemical. If the IC_{50} value is < 30 µg/ml, the sample is said to be very toxic to cancer cells, if the IC_{50} value is 30 - 1000 µg/ml, then the sample is toxic, and if the IC_{50} value is > 1000 µg/ml then the sample is not toxic to cancer cells. cancer cells. The IC_{50} results of the ethanol extract were 32.21, which is toxic, the n-hexane fraction were 616.59, is toxic, the ethyl acetate fraction were 90.57, is toxic, the n-butanol fraction were 338.84, is toxic and doxorubicin were 1.44, is very toxic.

The results of data analysis using one way ANOVA, on the concentration and percentage of T47D cell viability, from samples of ethanol extract, n-hexane fraction, ethyl acetate fraction and n-butanol fraction produced p value > 0.05, this means there is no significant difference between the four samples and doxorubicin injection, because all of them are toxic to T47D cancer cells. Likewise, with Duncan and Tukey's further analysis, there was no real difference between each sample in fighting T47D cancer cells, because they all had the same strength, namely being toxic.

4. CONCLUSION

The conclusion that the extract and fraction of arumanis mango rind had cytotoxic potential against T47D cancer cells, for the best of percentages of cell viability are ethanol extract and ethyl acetat fraction, the best of Inhibitory Concentration 50 (IC_{50}) are ethanol extract and ethyl acetate fraction, and the best of cancer cell morphology is ethanol extract and ethyl acetate fraction include doxorubicin as comparison drug.

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