INTRODUCTION

Cancer is one of the highest causes of death in the world. Colon cancer ranks number 3 with the highest incidence in Indonesia. Based on incidence, efforts should be made to prevent the spread of cancer as early as possible given the risks that can be caused. Cancer treatment using medical therapy requires high costs and has side effects. Soursop leaves have various contents of alkaloid, flavonoid, and acetogenin compounds. The spread of colon cancer cells begins with the process of moving cells to other tissues through the ability to migrate and invade cancer cells. This spread is the cause of the bad incidence of cancer, which can damage surrounding tissues and can metastasize to other organs. Seeing this incidence, it is necessary to make efforts to prevent the spread of cancer as early as possible considering the risks that can be caused.

Several ways of treating cancer using medical therapy are chemotherapy, radiotherapy, and surgery. However, given the large cost of treatment and the high level of risk that will be experienced after treatment, people use natural materials more as traditional therapy. Soursop leaf (Annona muricata L.) has been tested to be toxic to PC-3 prostate cancer cells, A549 lung cancer, MCF-7 breast cancer, HT-29 cell line colon cancer, and liver cancer cells. Soursop leaf (Annona muricata L.) is reported to be able to inhibit cancer in the proliferative phase such as activity and increase antiproliferative caspase against colon cancer cells. Annona muricata preparation that has been tested in the form of ethyl acetate leaf extract has been shown to have an effect on apoptosis, annomuricin E compound dose of IC 50 with 1.62 ± 0.24 g/ml after 48 hours is able to stop the G1 phase cell cycle, suppress the migration and invasion of HT-cell colon cancer. 29 and HCT 1166. However, the ability of soursop leaf (Annona muricata L.) to inhibit the migration of WiDr colon cancer cells as a derivative of HT-29 cells is not yet known. Based on the Cancer Chemoprevention Research Center, WiDr cells are often used for anti-chemotherapeutic analysis, so it is important to know the WiDr response to anticancer derived from soursop (Annona muricata L.) leaves.

The purpose of this study was to determine the effect of soursop ethanol extract (Annona muricata) leaves. L. in inhibiting the migration of colon cancer cells WiDr: the overview of wound assay method of soursop ethanol extract leaves in inhibiting migration of WiDr colon cancer cells. Combination Soursop (Annona muricata) with Camellia cinensis had anticancer properties.

ABSTRACT

Introduction: Soursop leaves have various contents of alkaloid, flavonoid, and acetogenin compounds. Soursop ethanol extract leaves (Annona muricata) showed many beneficials as antivirus, anti-hyperglycemic, anticancer, etc. As anticancer showed with cytotoxicity in some cancer cell line. But there was no research about wound assay in WiDr colon cancer cell line as a model of colon cancer diseases. This research carried out to explore the overview of wound assay method of soursop ethanol extract leaves in inhibiting migration of WiDr colon cancer cells.

Methods: The series of studies included extra ethanol cytotoxic tests of soursop leaves (Annona muricata L.) on WiDr colon cancer cells using MTT to assess cell viability and antimigration testing using the scratch wound healing assay method to assess the presentation of closure of WiDr colon cancer cell migration.

Results: Cytotoxic test of soursop ethanol extract leaves (Annona muricata L.) on WiDr colon cancer cells obtained IC50 values of 905.77 µg/ml and the cell antimigration test at 24 hours obtained the greatest migration inhibition, namely soursop ethanol extract leaves (Annona muricata L.) at a dose of ½ IC50 or equal to 452.85 µg/ml.

Conclusions: Soursop ethanol extract leaves (Annona muricata L.) has an effect on inhibiting migration of WiDr colon cancer cells so that it can be used instead of chemotherapy which can prevent and inhibit the growth of cancer cells.

Keywords: Annona muricata, WiDr, Migration

MATERIALS AND METHODS

**Extraction of Annona muricata Using Maceration Methods**

Sixty grams of *Annona muricata* leaf powder (60 g) was added to three 1000 mL Erlenmeyer flasks (20 g each) which were extracted using 200 mL of 95% ethanol solvent. For 24 hours the sample was extracted and shaken for 3 hours at room temperature. The dissolved fraction in ethanol was separated, put into a flask and the obtained pulp was macerated using the same solvent. This extraction was carried out three times. A total of 150 mL of immersion solvent was used in the second and third iterations. The filtrate obtained was concentrated with a vacuum rotary evaporator at a temperature of 60°C and stopped when the extract was sufficiently thick, indicated by being dissolved in a round bottom flask. The viscous extract is stored at a temperature of less than 20°C to prevent spoilage.

**WiDr Colon Cancer Maintenance**

Colon cancer WiDr from Cancer Chemoprevention Research Center, Faculty of Pharmacy, Universitas Gadjah Mada was maintained at RMPI, fertilized with FBS, fungizone, Streptomycin/penicillin. Cell growth up to 80% for treatment. To release cells, Trypsin and EDTA are used.

**Viability of WiDr Colon Cancer Cell Line Assay**

Viability test using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Reduction of yellow MTT tetrazolium salt to formazan crystals by the enzyme succinate dehydrogenase due to cellular metabolic activity by the enzyme succinate dehydrogenase is the principle of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. NAD(P)H-dependent cellular enzyme oxidoreductase in the cellular respiration path in mitochondria. NAD(P)H-dependent cellular enzyme oxidoreductase in the cellular respiration pathway in mitochondria. The count of a stopper reagent (ie detergent) will liquify these colored crystals which are then absorbed using an Enzyme-linked immunosorbent assay (ELISA) reader. The concentration of the purple color is proportional to the number of living cells, i.e. if the concentration of the purple color is greater, the number of cells is increasing. There were nine treatment groups as samples in the cytotoxic test. WiDr cells as a negative control neither given any treatment nor only permitted to grow in the growth media of the first group. Doxorubicin as a standard cancer drug at doses of 5 g/mL, 10 g/mL, and 15 g/mL was given to WiDr cells as positive controls to the second group. WiDr cells treated with *Annona muricata* leaves ethanolic extract at doses of 80 g/mL, 160 g/mL, 320 g/mL, 460 g/mL, and 800 g/mL were groups 3, 4, 5, 6, and 7. positive and negative controls were repeated three times in each treatment group. Meanwhile, the group of cells that were given the *Annona muricata* leaf ethanol extract was treated five times for each group.

**Migration Assay using Scratch Wound Healing Assay.**

It is estimated that 5x10^4 WiDr cell lines were seeded on the six-well plate. The cells were then divided into six treatment groups using negative control (cells with media only) and positive control (using doxorubicin). The treatment doses were 1/2 IC50, 1/3 IC50, 1/4 IC50, 1/8 IC50 ethanol extract of *Annona muricata* leaf. When the cells adhere and spread and then about 80% of the confluent grows, a streak is made using a sterile yellow tip. The scratch erases the cells then becomes a discrete area so that each edge cell can migrate to the empty area. It is necessary to add 5mL of treatment solution. Images were taken for each treatment at 12, 17, and 24 hours and then analyzed using Image J software. The migration test was repeated three times for each treatment. This research was recommended by the Ethics Committee with no:072/EC-EXEM-KEPK FKIK UMY/VIII/2021

**RESULTS**

To calculate the IC 50 (Inhibitor Concentration) based on data Table 1, then change to the linier equation as shown in Figure 1. The IC50 value indicates the concentration required to inhibit the

<table>
<thead>
<tr>
<th>Dose (µg/mL)</th>
<th>Percentage of Viability of WiDr Cell (%)</th>
<th>mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>89 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>160</td>
<td>88 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>320</td>
<td>78 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>640</td>
<td>66 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>800</td>
<td>53 ± 0.07</td>
<td></td>
</tr>
</tbody>
</table>

**Figure1.** The Equation of Viability Cell Number on Dose Depending Manner
growth of WiDr colon cancer cells by 50% of the total population. The IC50 value of soursop leaf ethanol extract (Annona muricata L.) was 905.77 µg/ml.

Linear regression analysis found EEAML dose significantly predict WiDr cell viability with R² of 0.9804. The linear equation predicting WiDr cell viability based on EEAML dose with is as follows:

\[ y = -0.049x + 94.83 \]

**Migration cell with wound healing assay**

Cell migration testing was carried out using the in vitro scratch wound healing method, which was observed at 0, 12, 17 and 24 hours after treatment. In the antimigration test, 2 treatments were given, namely doxorubicin as a positive control and soursop ethanol extract leaves (Annona muricata L.). The average of migration area shown on Table 2.

The significant area migration during observation on 12-, 17- and 24-hour period as shown in Figure 2. There was unsignificant on dose ½ IC50.

Meanwhile the migration area with wound assay as shown on Figure 3. The scratch areas swallow by the time on dose control and 1/2 IC50 faster than the other dose groups.

**DISCUSSION**

This research determined that extract ethanol *Annona muricata* leaf (EEAML) has IC50 908 ug/ml on WiDr Colon Cancer cell. This dose different from previous research that use *Annona muricata* seed from different place to different cell. The factor that influences of dose IC 50 such as type of land, weather etc. EEAML in this research have a moderate potential as a cytotoxic agent due to IC50 value obtained is less than 1000 g/mL. Meanwhile the categorized of very potent as a potential cytotoxic agent if the IC50 value around 10 - 100 g/ml.

The scratch wound healing method in this research carried out showed the ability of cells to migrate in vitro. The principle of this method is carried out by scratching the confluent monolayer cells using a sterile yellow tip to form scratches of a certain size. Cells will communicate with each other so that at a certain time they will close the scratches that have been made. Then during the migration process, observations were made by taking pictures using an inverted microscope periodically and comparing images to determine the presentation value of cell

### Table 2. The Average of migration WiDr colon cancer cell.

<table>
<thead>
<tr>
<th>Dose</th>
<th>12 hrs</th>
<th>17 hrs</th>
<th>24 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>31.90 ± 1.31</td>
<td>44.24 ± 1.76</td>
<td>53.00 ± 1.80</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>13.03 ± 3.47</td>
<td>14.56 ± 4.84</td>
<td>18.04 ± 7.90</td>
</tr>
<tr>
<td>½ IC50</td>
<td>18.55 ± 3.46</td>
<td>28.88 ± 11.72</td>
<td>44.98 ± 6.88</td>
</tr>
<tr>
<td>EEAM 1/8</td>
<td>17.48 ± 3.30</td>
<td>21.95 ± 2.06</td>
<td>37.87 ± 4.34</td>
</tr>
<tr>
<td>EEAM 1/4</td>
<td>15.17 ± 3.87</td>
<td>18.08 ± 3.78</td>
<td>32.70 ± 10.58</td>
</tr>
<tr>
<td>EEAM 1/3</td>
<td>12.57 ± 1.99</td>
<td>16.84 ± 1.32</td>
<td>21.28 ± 3.99</td>
</tr>
<tr>
<td>EEAM 1/2IC50</td>
<td>31.90 ± 1.31</td>
<td>44.24 ± 1.76</td>
<td>53.00 ± 1.80</td>
</tr>
</tbody>
</table>

**Figure 2.** Bar chart of the average migration (µM) at 12 hours, 17 hours, and 24 hours in WiDr colon cancer cells.

**Figure 3.** A, B, C, D are the migration of colon cancer cells WiDr at 0, 12, 17, and 24 hours after treatment.
migration closure. Table 2 showed the results of observing the percentage of coverage of the migration area of WiDr cancer cells which were analyzed by SPSS using the one-way ANOVA test to determine the significance level of giving soursop ethanol extract leaf (*Annona muricata* L.) to the ability to inhibit migration of WiDr colon cancer cells. The significance value of giving soursop leaf ethanol extract (*Annona muricata* L.) at the 12th, 17th and 24th hours respectively is < 0.000. The significance value indicates that the administration of soursop leaf (*Annona muricata* L.) ethanol extract has a significant difference so that it has an effect in inhibiting migration activity in WiDr colon cancer cells.

Figure 2. showed that the percentage of cancer cell migration closure in the control is greater than the treatment. This is because the control cells were not given any treatment. The smallest percentage of migration closure was shown in the 1/2 IC50 treatment, which means that it has the highest effect of inhibiting WiDr cancer cell migration closure among other concentrations of soursop leaf ethanol extract (*Annona muricata* L.). The smaller the percentage result, the greater the inhibiting activity in the migration of cancer cells.

At the 12th hour migration observation, the percentage of closure of WiDr colon cancer cells given soursop ethanol extract leaf (*Annona muricata* L.) with a concentration of 1/8 IC50, 1/3, IC50 respectively was 18.55 ± 3.46 %, 17.48 ± 3.30 %, 15.17 ± 3.87 %, and 12.57 ± 1.99 %. While the percentage of cell migration closure on doxorubicin administration with a concentration of IC50 was 13.03 ± 3.47%. In the 17th hour migration observation, the percentage of closure of WiDr colon cancer cells given soursop leaf ethanol extract (*Annona muricata* L.) with a concentration of 1/8, 1/3, IC50 was 28.88 ± 11.72%, 21.95 ± 2.06%, 18.08 ± 2.06% and 16.84 ± 1.32 %. Meanwhile, the percentage of cell migration closure on doxorubicin administration with a concentration of IC50 was 14.56 ± 4.84%. In the 24-hour migration observation, the percentage of closure of WiDr colon cancer cells given soursop leaf ethanol extract (*Annona muricata* L.) with a concentration of 1/8, 1/3, IC50 was 44.98 ± 6.88%, 37.87 ± 4.34%, 32.70 ± 10.58 %, and 21.28 ± 3.99%. Meanwhile, the percentage of cell migration closure on doxorubicin with a concentration of IC50 was 18.04 ± 7.90%.

The administration of IC50 concentration of soursop leaf ethanol extract showed the smallest percentage of migration area coverage compared to other soursop leaf (*Annona muricata* L.) ethanol extract concentrations. This shows that the ethanolic extract of soursop leaves (*Annona muricata* L.) with a concentration of IC50 has the highest inhibitory effect on the migration of WiDr colon cancer cells.

The effect of WiDr colon cancer cell migration after being treated with soursop leaf ethanol extract (*Annona muricata* L.) can be seen with an inverted microscope from the scratch area as shown on Figure 3. The closure of the scratch area on the control cells was faster than the closure of the scratch area on doxorubicin and soursop ethanol extract leaf (*Annona muricata* L.) because the control cells were not given any treatment. At 12 hours after treatment with ethanolic extract, soursop leaf (*Annona muricata* L.) showed an inhibitory effect on the area of cell migration which could be seen from the decrease in the percentage of migration area compared to control cells. It can be seen from Figure 4 that in the control cell migration area, there is a greater closure than the treatment with soursop ethanol extract leaf (*Annona muricata* L.). At the 17th hour after the treatment of soursop leaf (*Annona muricata* L.) it was shown that the extract was able to inhibit cell migration as indicated by the lower percentage of closure, while in control cells the migration activity increased, as evidenced by a very confluent closure compared to the treatment other. Doxorubicin treatment as a positive control showed an inhibitory effect on cell migration.

In this antimigration test, it can be seen that at a concentration of IC50 the ethanolic extract of soursop leaf (*Annona muricata* L.) has the highest percentage of migration inhibiting activity, which is 21.28% in WiDr colon cancer cells. This can prove that the ethanolic extract of soursop leaves (*Annona muricata* L.) has an effect in inhibiting the migration of WiDr colon cancer cells. Soursop leaves (*Annona muricata* L.) contain various compounds, namely flavonoids, alkaloids, essential oils and acetogenins. Soursop leaf (*Annona muricata* L.) has acetogenin compounds that can inhibit the migration of WiDr colon cancer cells due to its anticancer activity.

The acetogenin compound can inhibit the energy source for the growth of WiDr colon cancer cells. Inhibition of the power of energy causes cancer cells to not divide properly. The process of cell migration is hampered when acetogenin that enters the body attaches to cell wall receptors and functions to damage ATP in the mitochondrial wall so that energy production in cancer cells stops. It is suspected that the mechanism of action of the extract is through disruption of the mitochondrial membrane in resting cells in the G0/G1 phase, and through the induction of apoptosis.

In this study, doxorubicin was used as a positive control which was used as a cancer drug. The mechanism of doxorubicin as a chemotherapeutic agent in cancer treatment is DNA intercalation resulting in inhibition of DNA and RNA synthesis. The series of concentrations of doxorubicin used for the antimigration test was IC50 while the series of concentrations of the soursop ethanol extract leaf (*Annona muricata* L.) used for the antimigration test were 1/8, 1/3, and IC50. Migration percentage analysis was performed using Image software based on the size of the WiDr cancer cell migration area closure. The percentage of closure for each treatment at 0, 12, 17 and 24 hours was then compared with control cells.

Up to date, research on the active content of (*Annona muricata* L.) reported that there were as many as two hundred and twelve bioactive compounds. The most abundant compounds are alkaloids, phenols and other compounds. The part of (*Annona muricata* L.) that is reported to contain bioactive compounds is the leaves and seeds, because people often use...
In this study, it was observed that leaf of Annona muricata is capable of inhibiting the growth of cancer cells WiDr as a model of cancer cell. This research is a subsequent step to further research to prove the mechanism of inhibition migration or invasion as characteristics of cancer cells.

Soursop ethanol extract leaves (Annona muricata L.) has an effect on inhibiting migration of WiDr colon cancer cells. Soursop ethanol extract leaves (Annona muricata L.) can be used instead of chemotherapy which can prevent and inhibit the growth of cancer cells.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

There were not conflict of interest in this research.

FUNDING

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ETHIC APPROVAL

The protocol has been reviewed and approved by the appropriate ethics committee with approval letter no. 072/EC-EXEM-KEPK FKIK UMY/VIII/2021.

AUTHOR CONTRIBUTION

Yoni Astuti, contribute for Idea, design, analyzed of research and writing paper. Wahyu Joko Priambodo, preparing for ethanol extract of Annona muricata and analyzed data. Rahmah Rahmah, collection of data and manuscript preparation. Dara Ayu S, contribute on collecting, analyzing data.

REFERENCES