CYTOTOXICITY OF COMBINATION EXTRACT Pandanus conoideus Lam. Var. YELLOW AND RED FRUIT ON MCF-7 CELL

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**CYTOTOXICITY OF COMBINATION EXTRACT *Pandanus conoideus* Lam. var YELLOW AND RED FRUIT ON MCF-7 CELL**

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**ABSTRACT**

Many researchers have explored that natural substances have important compound of cancer drug. Variety of red fruit (*Pandanus conoideus* Lam.) has been extensively used to treat HIV/AIDS, diabetic mellitus, uric acid, osteoporosis and cancer. The sparse evidence leads to an uncertainty whether combination of yellow and red fruit extracts has positive or negative effect. The objectives of this research were to find out the cytotoxic activity of combination of *Pandanus conoideus*’s yellow and red fruit extract on MCF-7 cell line and the effect of this same extract on MCF-7 breast cancer cell line.

Method of Freshney was used in growing MCF-7 cell line. Cytotoxicity test was done to describe the presence of toxic effect on the living cell. Data was analyzed, according to Doyle and Griffith, by calculating the determination of cell death. The data obtained was analyzed using ANOVA followed by DMRT. The calculation of amount of living cell was done with the usage of Doubling time test.

Cytotoxicity test results demonstrated that the most active part with a dose of ½ LC BM dan 1LC BK were able to kill 59.9 % MCF-7 breast cancer cells, or showed the highest cytotoxic activity against MCF-7 cells. Concentration with a dose of 0.156: 0.625 µl / ml (BM: BK) proved most effective in inhibiting proliferation, with a longer cell doubling time of 9.135 times (from 11.44 hours to 104.51 hours).

**Keywords:** MCF-7, *Pandanus conoideus* Lam. var yellow and red fruit extract, cytotoxicity.
INTRODUCTION

Red fruit (*Pandanus conoideus*) is an endemic plant coming from Papua mostly growing wildly. Red fruit plant is located in Papua, Papua New Guinea, and Moluccas areas. This plant in its original habitat (Papua island) grows from the lower land to the higher land (Nehemiah, 2006). Red and yellow fruit, included into pandan plant (*Pandanus*). Estimated there are about 600 types of plants that are in the genus *Pandanus*, one them is red fruit (Sadsoeitoboen, 1999).

Based on the chemical analysis, the red fruit has completed nutrition content such as beta-carotene (700 ppm), tocopherol (11,000 ppm), and some essential fatty acid. Yellow fruit contains active compounds that are important as anticancer agent such as carotenoid (9500 ppm), β-carotene (240 ppm), and tocopherol (10,400 ppm) (Budi, 2000). In addition, this fruit also contains a little capric, lauric and miristic, linoleic, decanoic acids, omega 3, omega 6 and omega 9 (Tim Agromedia, 2005). Beta-carotene and tocopherol are known as antioxidant compound because they can prevent many diseases, beneficial to neutralize the free radicals and carcinogenic material (Nehemiah, 2006). Because it can bind the
free radicals, it can prevent the mutation from occurring and inhibit the tumor growth (Nehemiah, 2006).

Breast cancer is one disease mostly affecting the women throughout the world particularly in western countries (Coleman and Tsongalis, 2002; McPherson et al., 2000). Breast cancer belongs to malignant tumor coming from the epithelial cell layering the ductus and lobulus skin (De Vita et al., 1997). The underlying mechanism of breast cancer evolvement has not been known (Hedenfalk et al., 2002).

Until recently, people have tried to seek for other alternative treatment in addition to the use of chemotherapeutical agents. It included the use of natural material in addition to chemotherapeutical agents. Ideal natural material used as the co-chemotherapy is the one with synergic effect to the chemotherapy agent, so that the dosage of chemotherapeutical agent used can be lowered as an attempt of avoiding the side effect as well as of helping the cancer curing quickly (Jenie, 2008).

MCF-7 cell is one model of breast cancer cell widely used in research. MCF-7 is a breast cancer cell taken from the breast tissue of a 69 years-old Caucasian woman with O blood group, with Rh positive, constituting the adherent cell that can grow in the DMEM growth media or RPMI containing fetal bovine serume (FBS) 10% and 1% Penicilin-Streptomycin antibiotic (Anonymous, 2007). MCF-7 stands for Michigan Cancer Foundation – 7.

This research aims to find out cytotoxic activity and effect of combination of *Pandanus conoideus*’s yellow and red fruits extract on the MCF-7 cell.
MATERIALS AND METHOD

The main material employed in this research is the yellow and red fruit varieties extract of *Pandanus conoideus* Lam. obtained from I Made Budi (researcher *P. conoideus* Lam. from *Universitas Cendrawasih*, Papua). MCF-7 breast cancer cell culture obtained from American Type Culture Collection. Cell density is calculated by taking 20 µL cell suspension, and then added with 180 µL trypan blue. The total number of cell obtained is multiplied by the dissolution factor and $10^6$/ml number (Freshney, 2000). Cytotoxicity test was done by distributing MCF-7 cell ($2 \times 10^4$) distributed into wells on 96 microplate wells and incubated with a complete series of the test sample (namely 1LC yellow fruit + 1 LC red fruit, 1LC yellow fruit + ½ LC red fruit, ½ LC yellow fruit + 1LC red fruit (1 LC yellow fruit is 1.25 µl/ml and 1 LC red fruit is 0.625 µl/ml) for 24 hours.

Doubling time test was done by starving cells ($1.5 \times 10^4$) with culture medium containing 0.5% FBS, penstrep 2%, 0.25% trypsin and 0.5% fungizon for 24 hours. After one night DMEM media replaced and added new test solution with non lethal concentrations (below ½LC red fruit and 1LC yellow fruit that is 0.3125: 1.25 (µl/ ml)), each given concentration of 100µl in cell culture, repeated 3 times for each treatment. Non lethal concentrations are 0.156: 0.625 (µl/ml), 0.078: 0.312 (µl/ml), 0.039: 0.156 (µl/ml), 0.019: 0.078 (µl/ml) and negative control contains a cell suspension of DMEM media. The sample was taken at 0, 24, 48 and 72 hours. DMEM media was discarded and trypsin-EDTA as much as
100 µl was added and then incubated for 10 minutes and then added 100 µl tryphan blue.

RESULTS AND DISCUSSION

The morphology of control cells and after treatment can be seen in Figure 1. Microscopic observation showed the differences in cell morphology in MCF-7 controlled cells and cells with treatment (Figure 1). Perhaps this happened because of the cytoplasmatic membrane shrinkage. Control cells seemed shaped like leaves, attached at the bottom of wells, while cells treated with the concentration of ½ LC red fruit and 1LC yellow fruit looked dead. Dead cells appeared as changed shape, turbid and floats. Based on the research of Moongkarndi et al., (2004) it was determined there was change in cell morphology, after added the methanol extract of *Garcinia mangostana* pericarp on SKBR3 breast cancer cells, morphological changes were observed under phase contrast microscopy showed cytoplasmatic membrane shrinkage, loss of contact with the neighboring cells, membrane protrusion and cell apoptosis. Prakash et al., (2001) also showed that the qualitative analysis of lung cancer cells NCI-H69 indicated small morphological changes in this lung cancer cells treated with beta-carotene.

Based on Table 1 can be noted that the combination extract of *Pandanus conoideus* Lam. yellow and red fruit varieties kills breast cancer cells. Research was conducted to find out if *P.conoideus* Lam. yellow and red fruit varieties have cytotoxic activity against MCF-7 cells.
Table 1. Percentage of death resulted from cytotoxicity test of combination Extracts *Pandanus conoides* Lam, varieties of red and yellow fruit on MCF-7 (24 hours incubation).

<table>
<thead>
<tr>
<th>No</th>
<th>Concentration</th>
<th>Percentage of death</th>
<th>Mean (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Replication I</td>
<td>Replication II</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1LC RF + 1LC YF</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>1LC RF + ½LC YF</td>
<td>53,33</td>
<td>52,54</td>
</tr>
<tr>
<td>4</td>
<td>½LC RF + 1LC YF</td>
<td>58,90</td>
<td>60</td>
</tr>
</tbody>
</table>
Figure 1. The result of the appearance of MCF-7 cells in cytotoxicity tests on the magnification of 80 × after the addition of combination extract of *Pandanus conoideus* Lam.var Yellow and Red Fruit in treatment (a) control, (b) 1LC red fruit with 1LC yellow fruit, (c) 1LC Red Fruit with ½ LC Yellow Fruit (d) ½ LC Red Fruit with 1LC Yellow Fruit. (1LC red fruit with a concentration of 0.625 and 1LC Yellow Fruit with concentration 1.25).

Description: ○ ; living cells
○ ; dead cells
In Figure 2 is being shown that the percentage increase in mortality is proportional to the increase in concentration *P. conoideus* Lam varieties yellow fruit that were given. This suggests that the yellow fruit exert greater antioxidant activity than red fruit. According to Budi cit Astirin *et al.*, 2008 varieties of yellow fruits have a higher tocopherol content than the red fruit varieties, amounting to 10,400 ppm, while red fruit varieties containing only 9500 ppm tocopherol. Thus the results of this study demonstrate significance with the result of research of Astirin cit Budi *et al.*, 2008. Correlation between concentration of extract and MCF-7 cells is indicated by *r* which has a value of 0.452. The results of *R*\(^2\) is not close to 1, but is close to 0 indicating that there is no close relationship between the concentration combination red and yellow fruit that was used with the percentage of cell death, so that it can be seen that cell death is not caused by the treatment fraction. There is a possibility that cell death was caused by other factors such as pH or secondary metabolites in *P. conoideus* which caused another reaction when was mixed.
Description for the concentration:

1 = 1LC RF + 1LC YF
2 = 1LC RF + ½ LC YF
3 = ½ LC RF + 1LC YF
4 = Control

Figure 2. Graph of correlation between the percentage of MCF-7 cell death with concentrations *P. conoideus* Lam. red and yellow fruit varieties

Based on the data in Table 3 the slope value of control is 2086.813, while for concentration of 0.156: 0.625 µl/ ml (RF:YF) has a slope value of 52.063. The greater the slope value is the shorter the doubling time (Meiyanto *et al.*, 2003). This shows that combination of red and yellow fruits are capable of preventing/reducing nature of the proliferative MCF-7 cells. Greatest inhibition process occurs at a concentration of 0.156: 0.625 µl/ml (RF:YF) with a doubling time of 104.51 hours.
Table 3. Regression equation of incubation time and the number of cells, and the value of doubling time.

<table>
<thead>
<tr>
<th>Concentration (µl/ml) RF:YF</th>
<th>Regression equation</th>
<th>Slope value</th>
<th>R²</th>
<th>Doubling time value</th>
<th>Improvement to control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0,156 : 0,625</td>
<td>Y= 52,063x+5445,667</td>
<td>52,063</td>
<td>0,8</td>
<td>104,5</td>
<td>9,135</td>
</tr>
<tr>
<td>0,078 : 0,312</td>
<td>Y= 1253,458x-10832,667</td>
<td>1253,458</td>
<td>0,973</td>
<td>8,63</td>
<td>-</td>
</tr>
<tr>
<td>0,039 : 0,156</td>
<td>Y= 1427,083x-11555,667</td>
<td>1427,083</td>
<td>0,987</td>
<td>8,09</td>
<td>-</td>
</tr>
<tr>
<td>0,019 : 0,078</td>
<td>Y= 1892x-21778,333</td>
<td>1892</td>
<td>0,975</td>
<td>11,5</td>
<td>1,005</td>
</tr>
<tr>
<td>Control</td>
<td>Y= 2086,813x-23889</td>
<td>2086,813</td>
<td>0,961</td>
<td>11,44</td>
<td>0</td>
</tr>
</tbody>
</table>

CONCLUSIONS

Combination extract of *Pandanus conoideus* red and yellow fruits varieties showed cytotoxic activity and the highest growth inhibition against MCF-7 cells and the most active with a dose of ½ LC RF and 1LC YF could kill MCF-7 breast cancer cells 59.90% or showed the highest cytotoxic activity against MCF-7 cells. Combination of red and yellow fruit extract on MCF-7 breast cancer cells in vitro can reduce cell proliferation, which occurred extension of doubling time from 11.44 in control to 104.51 at a concentration of 0.156: 0.625 µl/ml (RF:YF). The concentration had a longer cell doubling time of 9.135.
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