IC₅₀ and Cell Viability of Combination of Ethanol Extract *Moringa oleifera* Leave (EEMo) and Ethanol Extract *Carica papaya* Leave (EECp) on Breast Cancer Cells

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**ABSTRACT**

This study aims to find out IC₅₀ and Cell Viability of the combination of ethanol extract leaves of *Moringa oleifera* (EEMo) and ethanol extract leaves of *Carica papaya* (EECp) on the growth of MCF-7 breast cancer cells culture. It was conducted in a true experimental laboratory using post-test only control group design method. The study showed that the effect of extract combination for MCF-7 Cell by using series concentration like 0, 20, 40, 80 and 160 µg/mL for 48, 72, and 96 hours with a cell density of 5x10⁴ after giving WST assay there is a decrease in the number of cell viability. Inhibition concentration of MCF-7 cell culture was also indicated by the IC₅₀ value which was included in the very strong category with details of each extract combination with 1:1 comparison the IC₅₀ value is 12.02 µg/mL.

**Keywords:** extract combination; IC₅₀; cell viability, MCF-7

**INTRODUCTION**

**Background**

Cancer is a disease that is controlled by uncontrolled cell division and the ability of cells to attack other biological tissues, either direct growth in neighboring tissues (invasive) or cells to distant sites (metastasis). The uncontrolled growth is caused by DNA damage that causes mutations in vital genes that control cell division. Cancer cells lose their control function towards recycling regulation and cell homeostasis function in multicellular organisms so that they cannot proliferate normally. As a result, cells will continue to proliferate continuously resulting in abnormal tissue growth.¹

Breast cancer begins when cells in the breast begin to grow out of control. These cells usually form tumors that are often seen on X-rays or felt as a lump. These tumors are malignant (cancerous) if cells can grow into (invade) the surrounding tissue or spread (metastasize) to distant areas of the body. Breast cancer occurs almost entirely in women, but men can develop breast cancer as well. Breast cancer can start from any part of the breast. Most breast cancers begin in the ducts that carry milk to the nipple (duct cancer). Some start in the glands that make milk (lobular cancer). The most common types of breast cancer are invasive ductal carcinoma, cancer cells growing outside the ducts to other parts of the breast tissue and invasive lobular carcinoma, cancer cells spread from the lobules to nearby breast tissue.²

Various studies on moringa leaves and papaya leaves (Carica papaya L.) respectively as compounds that provide anticancer effects.³⁻⁶ Moringa leaves as anticancer contain flavonoids, glyoxides, tannins, steroids/triterpenoids and papaya leaves as an anticancer contain alkaloid compounds, flavonoids, glycosides, and tannins.⁷ The combination of the two extracts for cancer treatment is an interesting thing to understand.
One of the cancer cell models that can be used in testing breast cancer therapy drugs is the MCF-7 cells (Michigan Cancer Foundation-7). MCF-7 cells are classified as cell line adherents\(^7\) which have characteristics including resistance to chemotherapy agents,\(^9,10\) expressing alpha estrogen (ER-\(\alpha\)), Bcl-2 overexpression,\(^11,12\) not expressing caspase-3,\(^13,14\) and resistant to doxorubicin.\(^10\) In this study, tests were conducted to determine the cytotoxicity of the combination of moringa leaf extract and papaya leaf extract on MCF-7 cells (breast cancer cell culture).

**METHODS**

**Tools**

- Analytical balance, vessel, rotary evaporator, waterbath, glassware, CO\(_2\) incubator, Cytotoxic Safety Cabinet, Enzym-Linked Immunosorbent Assay (ELISA reader), micropipette, hemocytometer, inverted microscope, Pasteur pipette, oven, 15 mL and 1.5 mL centrifuge, manual calculators, cameras and autoclaves.

**Materials**

- Moringa leaves and papaya leaves were taken from around Universitas Hasanuddin, MCF-7 cell culture and Vero cell culture obtained from Medical Research Center (HUMRC) Universitas Hasanuddin, Dulbecco's Modified Eagle Medium (DMEM), ethanol 96% pa, ethanol 70%, Dimethyl Sulfoxide (DMSO), WST Reagent, trypan select, trypan blue, phosphate buffered saline (PBS), Penicillin-streptomycin, Sodium Dodecylsulfate (SDS), 96-well plate.

**Extraction**

- Simplicia moringa leaf and papaya leaf were weighed and macerated for 3 times 24 hours with 96% ethanol and assisted by stirring at room temperature. The maceration results are filtered and then evaporated with a rotary evaporator. The extract was left on a water bath until the extract becomes thick.

**Phytochemical screening ethanol extract**

- Phytochemical screening carried out on each extract (ethanol extract of Moringa leaves and ethanol extract of papaya leaf) examining the chemical secondary metabolites like alkaloids, flavonoid, tannins, saponins, and triterpenoids.

**Preparation of Test Solutions**

- Ethanol extract of moringa leaf and ethanol extracts of papaya leaf were each weighed as much as 10 mg and dissolved in 200 \(\mu\)L DMSO and 800 \(\mu\)L DMEM media with the help of vortex to obtain various concentrations, those were 20, 40, 80 and 160 \(\mu\)g/mL for all types on the single extract or combination extract. The combined extract was made by 1:1 comparison category (Moringa extract: Papaya extract).

**Cytotoxic Test**

- \(\text{IC}_{50}\) and cell viability test on MCF-7 cell culture was carried out using the WST assay. Cell cultures were grown on 96-well plates (100 \(\mu\)L for each well) with a cell density of 5 \(\times\) 10\(^5\). Cells were incubated for 24 hours and then the medium was discarded and rinsed using PBS. Cells in each well were treated by adding a series of concentrations of single and combined extracts (20, 40, 80 and 160 \(\mu\)g/mL) with a volume of 100 \(\mu\)L for control and replicated three times then incubated for 48 hours. After 48, 72, dan 96 hours, it was removed from the incubator and then added 5 \(\mu\)L of WST reagent and allowed to stand at 37\(^\circ\)C for 4 hours in a CO\(_2\) incubator then baked for 1 minute. The absorbance was measured using an ELISA reader at a wavelength of 450 nm.

**Data Analysis**

- The data obtained from the ELISA reader were analyzed using Graphpad Prism software to calculate the \(\text{IC}_{50}\) value and to calculate the cell viability the formula was used as follows:

\[
\text{% Viability} = \frac{\text{absorbance of treatment} - \text{absorbance of medium}}{\text{absorbance of control cells} - \text{absorbance of medium}} \times 100
\]
RESULTS

This research was conducted experimentally to determine whether the ethanol extract of moringa leaves (EE\textsubscript{Mo}) and the ethanol extract of papaya leaves (EE\textsubscript{Cp}) had a combined effect on MCF-7 breast cancer cells. The research stages included the collection and manufacture of simplicia, the manufacture of the extract and the extract phytochemical screening. Finding IC\textsubscript{50} and Cell viability of EE\textsubscript{Mo} and EE\textsubscript{Cp} as single and combined extracts. The implementation of this research took place from January 2020 to February 2020 at the Biology Laboratory of Universitas Negeri Makassar and Medical Research Center (HUMRC) of Universitas Hasanuddin Makassar. Preparation of phytochemical extracts and screening was carried out at the Phytochemical Laboratory of the Faculty of Pharmacy, Universitas Hasanuddin.

Phytochemical screening of moringa leaf ethanol extract (EE\textsubscript{Mo}) and papaya leaf ethanol extract (EE\textsubscript{Cp}) was carried out to determine the secondary metabolite compounds contained in each extract, the results of the phytochemical screening of ethanol extract of each plant can be seen in table 1.

Table 1. Results of phytochemical screening of ethanol extract leaves of \textit{Moringa oleifera} (EE\textsubscript{Mo}) and ethanol extract leaves of \textit{Carica papaya} (EE\textsubscript{Cp})

<table>
<thead>
<tr>
<th>No</th>
<th>Secondary metabolite compounds</th>
<th>EEMo</th>
<th>EECp</th>
<th>Reagent</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Flavonoid</td>
<td>+</td>
<td>+</td>
<td>AlCl\textsubscript{3} 10%</td>
<td>Result (+) yellow spots after spraying 10% AlCl\textsubscript{3}</td>
</tr>
<tr>
<td>2.</td>
<td>Tannin</td>
<td>+</td>
<td>+</td>
<td>FeCl\textsubscript{3} 5%</td>
<td>Result (+) black spots after spraying with FeCl\textsubscript{3} 5%</td>
</tr>
<tr>
<td>3.</td>
<td>Triterpenoid</td>
<td>+</td>
<td>+</td>
<td>H\textsubscript{2}SO\textsubscript{4}</td>
<td>Result (+) pink spots after spraying H\textsubscript{2}SO\textsubscript{4}</td>
</tr>
<tr>
<td>4.</td>
<td>Alkaloid</td>
<td>+</td>
<td>+</td>
<td>Mayer, Dragendorf</td>
<td>Yield (+) White precipitate after adding Mayer reagent, and red precipitate after adding Dragendorf</td>
</tr>
<tr>
<td>5.</td>
<td>Saponin</td>
<td>-</td>
<td>-</td>
<td>Aquadest</td>
<td>Result (-) No stable foam was formed after heating and shaking</td>
</tr>
</tbody>
</table>

Information:

(+) = there is a compound
(-) = there is no compound

Cell culture MCF-7 tested using the WST assay method against various concentration series showed that there was a tendency to decrease cell viability, namely the higher the extract concentration, the less the percentage of living cells, while the cytotoxicity test using the WST method on Vero cell culture showed a tendency to increase in viability. cells based on various concentrations of a given extract. The IC\textsubscript{50} values and viability of EE\textsubscript{Mo} and EE\textsubscript{Cp} cells against MCF-7 cells in single and combined extracts can be seen in table 2 and table 3.

Table 2. IC\textsubscript{50} Values of Single Extract and Combination Extract

<table>
<thead>
<tr>
<th>No.</th>
<th>Type of extract</th>
<th>IC\textsubscript{50} (\mu g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MCF-7</td>
</tr>
<tr>
<td>1.</td>
<td>Single extract of EEMo</td>
<td>28.45</td>
</tr>
<tr>
<td>2.</td>
<td>Single extract of EECp</td>
<td>25.60</td>
</tr>
<tr>
<td>3.</td>
<td>Combination Extract 1:1</td>
<td>12.02</td>
</tr>
</tbody>
</table>

*Combination Extract (EE\textsubscript{Mo}: EE\textsubscript{Cp})
Table 3. Cell viability values of single extract and combination extract EEMo and EECp of MCF-7 cells and Vero cells

<table>
<thead>
<tr>
<th>No</th>
<th>Concentration of Extract</th>
<th>Log Concentration</th>
<th>EEMo MCF-7</th>
<th>Vero</th>
<th>EEMo MCF-7</th>
<th>Vero</th>
<th>EECp MCF-7</th>
<th>Vero</th>
<th>EECp MCF-7</th>
<th>Vero</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0</td>
<td>0.0000</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2.</td>
<td>20</td>
<td>1.3010</td>
<td>105</td>
<td>182</td>
<td>74</td>
<td>228</td>
<td>87</td>
<td>191</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>40</td>
<td>1.6021</td>
<td>89</td>
<td>141</td>
<td>71</td>
<td>156</td>
<td>86</td>
<td>171</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>80</td>
<td>1.9031</td>
<td>84</td>
<td>117</td>
<td>66</td>
<td>170</td>
<td>81</td>
<td>150</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>160</td>
<td>2.204</td>
<td>83</td>
<td>160</td>
<td>48</td>
<td>181</td>
<td>80</td>
<td>133</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Combination Extract (EEMo: EECp)

Based on the IC50 value for single extract and combination extract in MCF-7 cell culture (Table 2) is below 50 µg / mL, the antioxidant activity is very strong category with the smallest number in the combination extract (1: 1) which is equal to 12.02 µg / mL while for In Vero cell culture, the smallest IC50 value was the single extract of moringa, which was 79.59 µg / mL which was included in the strong category. (16) The cell viability value of MCF-7 cells showed a decreasing trend while in vero cells showed an increasing tendency. The cell viability was then observed to see the proliferation that occurred. The administration of single and combined extracts at all concentrations showed that MCF-7 cell culture inhibited the proliferation process but there are several other important findings that will be presented in the following graphs.

Figure 1. Comparison chart of the proliferation kinetics test between MCF-7 cell culture and Vero cell culture with a single papaya extract treatment.

Figure 2. Comparison chart of the proliferation kinetics test between MCF-7 cell culture and Vero cell culture with single moringa extract treatment.

The observation of the proliferation kinetics for the percent of live cells MCF-7 in the control cell treatment as a comparison from 48, 72 and 96 hours, the number of live cells remained at 100%. If we compare the percent of live cells for the three sampling times (48, 72, and 96 hours), it will be seen that the percent survival of cells at 72 hours for all treatments except 160 µg / mL and decreases at 96 hours indicates that
proliferation, occurred at 72 hours after planting and its proliferation was inhibited at 96 hours. In the treatment of giving extracts for all concentrations in Vero cell culture, the proliferation from the lowest to the highest concentrations (20, 40, 80 and 160 µg / mL) at 72 and 96 hours decreased. the number of living cells compared to the percent living cell yield for the 48th hour.

The observation of the proliferation kinetics for the percent of live cells MCF-7 in the control cell treatment as a comparison from 48, 72 and 96 hours, the number of live cells remained at 100%. When compared the percent of live cells for the three sampling times (48, 72, and 96 hours) there was an increase in the percent live cells at 72 and a decrease at 96 hours for all treatments except for a concentration of 20 µg / mL. The administration of the extract in Vero cell culture showed that the proliferation at 72 hours with a concentration of 20 µg / mL and 160 µg / mL decreased but at concentrations of 40 µg / mL and 80 µg / mL there was an increase while at the 96th hour there was a decrease in cells. live for all concentration.

![Combination extract 1:1](image)

**Figure 3.** The comparison chart of the proliferation kinetics test between MCF-7 cell culture and Vero cell culture with a 1:1 combination extract treatment.

The administration of a combination extract with a ratio of 1:1 between papaya extract and moringa extract showed that at all treatment concentrations from the lowest to the highest (20, 40, 80 and 160 µg / mL) for MCF-7 cell culture at 72 hours and hours. 96 experienced an increase in the number of living cells by about 1% -12%, whereas in Vero cell culture there was a decrease in the number of living cells even for concentrations of 160 µg / mL it decreased by 54% and 58%.

**DISCUSSION**

The use of papaya and moringa leaves has been widely used by the public. The antioxidant content of each of these herbal ingredients has been empirically proven to cure cancer. The purpose of this study was to scientifically prove the effectiveness of the combination of Moringa leaf extract and papaya leaf extract in inhibiting the growth of MCF-7 cell cultures. Papaya (Carica papaya L.) contains alkaloid and phenolic anticancer compounds. Several cancer cells that can be inhibited by papaya leaf extract have been investigated, namely stomach cancer cells (AGS), pancreatic cancer cells (Capan-1), cancer cells. colon (DLD1), ovarian cancer cells (Dov-13), lymph cancer cells (Karpas), breast cancer cells (MCF-7), neuroblastoma cancer cells (T98G), uterine cancer cells (HeLa), T-cell leukemia cancer cells (CD26 negative). In another study, the IC$_{50}$ of papaya leaf extract against squamous cell carcinoma was 77.18 µg / mL. The anticancer activity of papaya leaves has been previously studied on HeLa cells obtained IC$_{50}$ 40.868 µg / mL. Other research on the leaves is that the leaf extract is able to inhibit the growth of cervical, liver, and leukemia cancers by inhibiting the proliferation of hematopoietic cells and solid tumors.

The results of the EEDK and EEDP phytochemical screening tests showed that each plant ethanol extract contained secondary metabolites in the form of alkaloids, flavonoids, tannins and triterpenoids but no saponins were found (Table 1). Alkaloids are very important compounds in plants where alkaloids have anticancer activity. The alkaloid group can cause damage and shrinkage of the cell membrane so that the constituent components of the membrane will change and the physiological process of the membrane will be disrupted. Flavonoids are phenyl compounds that are substituted by benzopyran derivatives which consist of a C15 (C6-C3-C6) basic framework. Several plants containing flavonoid derivatives have been used as disease prevention and therapeutic agents in traditional medicine in Asia for thousands of years, including as anticancer agents. The presence of flavonoids in EEDK and EEDP suggests the possibility of these extracts having anticancer effects. It was found that kaempferol, quercetin and isorhamnetin compounds in moringa leaves, these
compounds are flavonoids that have good anticancer effects which play a role in inhibiting cell proliferation and inducing cell apoptosis.(23) Triterpenoids are compounds that have high antitumor activity that have been tested through its ability to block nuclear factor-kappa B, where Nf-KB can inhibit apoptosis, activate transcription and angiogenesis.(24) Tannins are a secondary metabolite compound found in plants and synthesized by plants.(25) Tannins are compounds that have a molecular weight of 500-3000 and contains a large number of phenolic hydroxy groups which allow effective cross-linking with proteins and other molecules such as polysaccharides, amino acids, fatty acids and nucleic acids.(26) Four secondary metabolites are present in each of them, the extracts interact with each other and inhibit the growth rate MCF-7 cells however do not inhibit Vero cells.

The absorbance value obtained from the ELISA reader reading was used to determine the linear regression equation in order to obtain the IC$_{50}$ value of single papaya leaf extract, single moringa leaf extract, and papaya-moringa leaf extract combination. IC$_{50}$ is the concentration of the test compound capable of scavenging free radicals by 50%. In this study, it was shown that increasing the concentration of the extract could decrease cell viability with an IC$_{50}$ value of 25.60 µg/mL so that the ethanol extract of 96% papaya leaves had cytotoxic activity (Table 2). This study is in line with research conducted by Amalia(27) that the papaya leaf extract (Carica Papaya L.) has an IC$_{50}$ against MCF-7 cells of 9.73 µg/mL. The same thing was seen in the use of the ethanol extract of Moringa leaves that increasing the concentration of the extract could cause a decrease in the percentage of cell viability of MCF-7 cell culture with an IC$_{50}$ value of 28.54 µg/mL. (Table 2). Based on the IC$_{50}$ value for single extracts and combination extracts in MCF-7 cell culture (Table 2) is below 50 µg/mL, the antioxidant activity is very strong, while for Vero cell culture, the IC$_{50}$ value shows several categories, namely for single extracts of moringa are in the range with a value of 50-100 µg/mL, the antioxidant activity is in the strong category, while for single papaya extract and combination extract with a ratio of 1:1 is in the value range of 100-150 µg/mL, then the antioxidant activity is in the moderate category.(28)

The cell viability value (table 3) in the single extract test and the combined extract in MCF-7 cell culture showed that the higher the concentration of the extract given, the less number of living cells <100%. This shows that the higher the single extract and the combined extract given, the higher the cytotoxic effect on MCF-7 cells while different things were found in the viability value of Vero cell cultures. In Vero cell culture, there was an increase in the number of cell viability values >100%, meaning that there was cell growth even though there were variations in the number based on differences in extract concentrations. The trend is seen that the extract with a high concentration of 80 or 160 µg/mL the number of cells increases less than that of the smaller extract concentration.

**CONCLUSION**

The findings of this study can be understood as the combination extract of ethanol extract moringa leave and ethanol extract papaya leave was inhibited the process of the MCF-7 cell culture growth showed by decrease cell viability with IC$_{50}$ 12.02 µg/mL.

**REFERENCES**


