Chapter 1:

INTRODUCTION

2.1 Background

Cancer is one of the diseases that leading to the cause of death in worldwide. Based on the WHO database, in 2018, there were around 9.6 million of deaths mostly at the last stage of cancer. The causes are not just from the genetic factors but external factors also play roles in inducing the risk of cancer. World Health Organization (WHO) categorizes 3 external factors, such as physical carcinogens, chemical carcinogens, and biological carcinogens. Other than that, in developed countries, there are still many people with a low level of healthy lifestyle which also triggers the increasing number of the cancer cases.

Nowadays, chemotherapy is still the first-line treatment in cancer by administering chemicals or drugs to kill the cancer cells and has a systemic effect (Huang et al., 2017). Mostly the anticancer damage will affect the DNA synthesis such as damaging the DNA, interfering the enzymes needed for DNA to undergo replication and transcription, and moreover they will involve in the cell cycle (Huang et al., 2017). Other than the chemotherapy, immunotherapy can give a promising breakthrough to overcome the cancer by the self-immune system stimulation. Unfortunately, it needs higher cost than the chemotherapy or other common treatments for cancer.

Chemotherapy showed unwanted side effects such as hair loss, anemia, bruising and bleeding, loss of appetite that mostly fatigue, and vomiting and because of that, then many patients try to use alternative ways, one of them is by consuming traditional medicine. *Phyllanthus niruri Linn* is one of the herbs that can be used for the traditional medicine and suspected has antiproliferative activity. It is a plant of a genus that mostly can grow in the tropical and subtropical countries (Tang and Sekaran, 2011). In Indonesia, this plant is called as "Meniran" and usually grows along the gutter or along the place that has high humidity.

Moreover, in Indonesia as the developed country, the technologies that are used to support the cancer treatment still not advance together with the low income per capita and lack of education of the citizens. Because of that, some people choose to get the treatment from other countries such as Singapore, America, and some European countries or deciding to take the alternative ways although the success rates are also low. One of the alternative ways is by using herbs, unfortunately, one of the plants which is the *Phyllanthus niruri* is still need to be investigated more before it can be claimed as anticancer drug. It needs more analysis about its molecular mechanisms towards various genes where mostly mutated in cancer cases, the compounds that can affect the growth of the cancer cells together with the side effects and the doses that can be administered to the patients.

1.2. Objectives

The objective of this experiment is to observe the effects of IC50 concentration of *Phyllanthus niruri* extract toward the *p53*, *Bax*, B-cell lymphoma-2 (BCL-2), Retinoblastoma (*Rb*) gene expressions related to the HeLa cell proliferation inhibition and apoptotic pathway in molecular level. It is because *p53* is one of the key genes leading to apoptosis, controlling the cell cycle, restraining tumor arrangement, maintaining cell genome integrity and reacting to cell stresses. While BCL-2 gene can prevent the cells from apoptosis. This research will use HeLa cell and normal cell line as the comparison to see any changes in the gene expressions especially the apoptotic gene.

1.3. Scope of Work

This experiment will focus on the HeLa cell culture treated by the *Phyllanthus niruri* extract and will be analyzed with the qRT-PCR to see the genetic changes related to the antiproliferation. The steps are including:

- Cell thawing
    - Obtaining the cell line from −80°C, warm it, and place into the T-25 flask

- Cell passaging
    - Transferring the HeLa cells from the old media to the new media in T-25 flask based on its confluency (60% or more)
- Cell counting
    - Calculating the cells needed taking from the flask containing cells that has been confluent for seeding before the starving and treatment
- Cell seeding
    - Transferring desired amount of the cells into the 6-well plate and incubating it for 24 hours before the starving for the treatment
- *Phyllanthus niruri* extract preparation
    - Making the stock and then doing the serial dilution until get the desired concentration
- Cell treatment
    - After the seeding and starving, the media of the cells will be changed with the DMEM only mixed with the desired concentration of the *Phyllanthus niruri* extract.
- RNA extraction

Purifying the RNA taken from the HeLa cells, checking the purity by using nanodrop and the integrity by using the gel electrophoresis. Calculating the RNA at least 100 ng/µl for the DNA synthesis preparing for qRT-PCR analysis with the following steps:

- Cell pellet collection
- Sample homogenization
- Phase separation
- RNA precipitation

- RNA wash
- RNA resuspension
- Gel electrophoresis
    - Using the agarose gel to assess the RNA integrity involving their electricity charges
- cDNA syhnthesis

- qRT-PCR
    - Determining the temperature for denaturation, annealing, and extension based on the melting temperature of the primers
    - Determining the threshold and ct value by using Q-Rex software
    - Calculation of the fold change or the gene expression by using Microsoft Excel through the ΔCt value, ΔΔCt value, and $2^{-\Delta\Delta Ct}$ value calulation

Those scopes of work are followed with some questions:

- Does the extract give a toxic effect to the healthy cells?
- Does the extract can induce the proapoptotic genes which can lead to the cell death in the cancer cell line?