CHAPTER I
INTRODUCTION

A. Background of Study

Cancer is one of the perilous diseases and causes the main health problems in human. Cancer is abnormal proliferations of cells coupled with invasive characteristics into surrounding tissue or distant organs in patients. Cervical cancer or uterine cervical carcinoma is the second most common type of cancer in women worldwide after breast cancer and is considered as one of the deadliest gynecologic cancers occured in women. In 2020, nearly 604,127 women were diagnosed with cervical cancer and 341,831 of them died. In the same year, Asia (including east, southeast and south-central Asia) topped the list for the highest number of cases or deaths caused by cervical cancer (Ferlay et al., 2021). According to estimate from the American Cancer Society on cervical cancer for 2023, about 13,960 new cases of invasive cervical cancer will be diagnosed and 4,310 women will die from cervical cancer in the United States. Cervical pre-cancers are diagnosed far more often than invasive cervical cancer (American Cancer Society, 2023).

In Indonesia, cervical cancer has received enormous attentions and brought up particular concerns by the Indonesian Ministry of Health. More than 93 million Indonesian women are at risk of developing this disease and around 21,000 new cases are diagnosed annually (Spagnoletti et al., 2019). According to WHO, about 2 out of 10,000 Indonesian women suffer from cervical cancer and an estimated 26 women die from cervical cancer every day (Kemenkes RI, 2016). Recently, according to data from Globocan, International Agency for Research on Cancer (IARC), the incidence of cervical cancer in Indonesia is around 32,469 cases (17.2%) with a mortality rate of 18,279 (8.8%) in 2018 (Sung et al., 2021). Based on this, cervical cancer is one of the largest cancer contributors of all types of cancer. This large number of cases and deaths is caused by population expansion, lack of screening and vaccination facilities, and the high cost of cancer treatment (Garland et al., 2012).

There is no single cause for certain types of cancer, and cancer is therefore considered a multifactorial disease involving both genetic and environmental

factors. Genome integrity is tightly regulated by the cell cycle to stop genetic changes being passed on to the next generation (Lukas et al., 2004). However, deregulation of cell cycle checkpoints including G1/S and G2/M phases has been documented to be associated with malignancy (Foster, 2008). Cell cycle arrest provides an opportunity for DNA repair to occur, thus inhibiting the replication of the damaged template (Murray, 2004). Hence, it is considered as one of the effective strategies to eliminate cancer cells (Buolamwini, 2000).

Besides the cell cycle, apoptosis or programmed cell death is also another key strategy to eliminate cancer cells (Shankar, 2008). It acts as a protective mechanism that destroys potentially harmful or damaged cells prior to malignancy manifestation, without activating an inflammatory response (Singh et al., 2011). Many chemotherapy drugs including cisplatin, tamoxifen and doxorubicin cause cell cycle arrest and induce apoptosis in eliminating neoplastic cells (Lüpertz et al., 2010). Despite the effectiveness of current cancer chemotherapy drugs, adverse side effects have prompted a demand for the development of new therapeutic agents.

Various therapeutic strategies have been developed in the treatment of cervical cancer, including surgery, radiotherapy, chemotherapy, or a combination of the three according to the stage of the cancer. Failure of cancer treatment, such as surgery performed by removing cancer tissue, still has the risk of growing into new cancer tissue due to incomplete removal of cancer tissue. Giving chemotherapy and radiotherapy to cancer patients often causes negative effects in the form of nausea, vomiting, leukopenia, and stomatitis (Mangan, 2009). Several types of drugs have been used for chemotherapy in the treatment of cervical cancer, but they often cause unwanted and detrimental side effects to patients such as neutropenia, thrombocytopenia, anemia, gastrointestinal disorders, menstrual changes, and hair loss (American Cancer Society, 2020). To overcome the problem of cancer treatment, we need anticancer agents that are effective and cost-effective with minimal or limited side effects. In this task, drugs derived from natural products are preferred because they are claimed as important sources for drug lead candidates.

Simpor plant (*Dillenia suffruticosa*) is one of the shrub plants spread in Australia and Asia. In Bangka Belitung Province, simpor leaves are used by the community as wrappers for traditional foods such as lontong, lakso, pepes, fish

wrap, and tempeh. In addition, simpor plants are also used as medicine for treatments of various diseases such as wounds, rheumatism, fever, and cancer (Saiful Yazan & Armania, 2014). Simpor is already known for its potential as an anticancer agent. Methanol extract from the roots of the simpor plant originating from Terengganu, Malaysia exhibited significant antioxidant properties and cytotoxic activity especially against cervical cancer cells (HeLa) (Armania, Yazan, Ismail, et al., 2013). Studies conducted by Armania, Yazan, Ismail, et al. (2013) have shown that phenolic content is an important contributor to the high antioxidant activity that has been observed in methanol root extracts of *D. suffruticosa* species. Although these extracts showed the highest antioxidant and cytotoxic activity in HeLa cell lines, it was found that dichloromethane and ethyl acetate extracts showed higher cytotoxicity in breast cancer cell lines, MCF7, MDA-MB-231, lung cancer *cell line* A549 and colon cancer cell line HT29 (Armania, Yazan, Ismail, et al., 2013). The cytotoxic activity of dichloromethane extract of *D. suffruticosa* can be attributed to the presence of phytochemicals such as saponins, triterpenes, sterols, and polyphenolic compounds (Armania, Yazan, Ismail, et al., 2013). Saponins are collective terms for triterpenoids and steroidal glycoside glycosides (Rao & Gurfinkel, 2000), which among 150 types of natural saponins have shown significant anticancer activity (Man et al., 2010). Polyphenols also which include the same phenolics, flavonoids, stilbens, and lignans have been known for their many beneficial health benefits, among which there are anticancer, antiviral, antidiabetic, anti-aging, and neuroprotective activities (Pandey & Rizvi, 2009).

It has been demonstrated that extract from the simpor plant inhibits the proliferation of HeLa cervical cancer cell line, MCF7 and MDA-MD-231 breast cancer cell lines by induced the cell cycle arrest in the G2/M phase and apoptosis (Armania, Yazan, Ismail, et al., 2013; Armania, Yazan, Musa, et al., 2013). Moreover, extracts from the roots of simpor plant by methanol, ethyl acetate and dichloromethane have very potential cytotoxic activity against cancer cells MCF-7, MCF10A, HT29, CaOV3, and HeLa (Armania, Yazan, Ismail, et al., 2013; Armania, Yazan, Musa, et al., 2013; Foo et al., 2014, 2016).

Simpor plants in the Bangka Belitung area are generally used by the villagers to wrap food, and so far, there has been no research found on the potential of the

ethanol simpor leaf extract from Bangka Belitung to treat cancer. Therefore, in this study, we would like to assess the cytotoxic effects and apoptosis induction of alcohol extraction of *D. suffruticosa* leave (AEDSL) against to cervical cancers. It is known that the phytochemical compounds that greatly contribute to the cytotoxic activity carried out by simpor extract on hela cells are saponins, triterpenes, sterols, and polyphenolic compounds (Armania, Yazan, Ismail, et al., 2013). Most of those compounds are polar, therefore a polar solvent is also needed to make it easier to attract these compounds. Ethanol is a polar solvent, which is why it is used in this study. HeLa cells are often used for research because of the immortal and productive nature of cells when cultured. Moreover, HeLa cells are a model cell line that is often used in the biomolecular field, with more than 70,000 publications using HeLa cells (Cherry et al., 2013). To characterize the great potential anticancer effects of the alcohol exractions of *D. suffruticosa* from Belitung, we determined the proliferation and apoptosis of AEDSL-treated HeLa cervical cancer cells using the flow cytometry and MTT assessments. It may benefit the large number of cervical cancer patients worldwide as well as Indonesia patients.

B. Problem Statement

Based on the background, the formulation of the problem taken in this study is as follows:

1. How much TFC and TPC that were contained in the ethanol extract of simpor leaves (*Dillenia suffruticosa*)?
2. How is the toxicity of simpor (*Dillenia suffruticosa*) leaf extract against the HeLa cell line?
3. What are the changes in the morphology of the HeLa cell line after being treated with simpor (*Dillenia suffruticosa*) leaf extract?
4. How is the ability of simpor (*Dillenia suffruticosa*) leaf extract to induce apoptosis in HeLa cell lines in vitro using Annexin V with flow cytometry method?

C. Research Objectives

Based on the formulation of the problem, this study aims to be as follows:

1. To determine the quantity of TFC and TPC contained in simpor (*Dillenia suffruticosa*) leaf extract.
2. To determine the LC_{50} and IC_{50} toxicity of simpor (*Dillenia suffruticosa*) leaf extract using the BSLT and MTT assay.
3. To assess the morphological changes in cell line descriptively.
4. To analyze the ability of simpor (*Dillenia suffruticosa*) leaf extract in inducing apoptosis in HeLa cell culture in vitro using Annexin V with flow cytometry method.

D. Research Benefits

The results of this study are expected to provide benefits including:

1. To investigate the ingredients of simpor plants for the drug development of cancer therapy.
2. To contribute to identifying drugs for cervical cancer.
3. To become one of the supporting references for other researchers to conduct further research on related matters.