

CHAPTER 1: INTRODUCTION

1.1 Project Background

Cancer is the second leading cause of death which is responsible for approximately 9.6 million cases of death in the world (WHO, 2018) and contributes to approximately 350.000 new cases per 2018 with more than 200.000 death cases in Indonesia based on the Global Cancer Observatory. Chemotherapy is one of the common therapeutic options to treat cancer (Singh & Singh, 2018). Chemotherapy targets and inhibits rapidly proliferative cells such as cancer by altering the DNA, RNA, and protein synthesis, which is a crucial pathway for their growth and survival (Amjad & Kasi, 2020). Nevertheless, a chemotherapeutic agent is cytotoxic not only to cancer cells but also to a highly replicative cell under the normal physiological condition, such as hair follicles cell, GI tract mucosa and bone marrow. In consequence, chemotherapy causes chemotherapeutic-induced adverse effects as for instance, hair loss, nausea/vomiting, fatigue and also pain (Bhatnagar & Satija, 2017).

Outstanding attention has been given to natural based-medicine that originates from plants due to its biologically potent phytochemicals contained in the plant that can potentially be used as a treatment option for cancer. Natural products have been widely used by humankind to fulfill their nutritional needs at first. Following the discovery of therapeutic potential properties within the natural product, especially plants, people start to use them as a source of medicine (Azmir et al., 2013). Natural based-medicine is lower in adverse effect compared to the available chemotherapeutics agent in the market since chemotherapy is able to induce a detrimental effect that could possibly reduce the patient's quality of life (Zhang et al., 2018). Besides, its cost-effectiveness and its availability are other factors that are considered by many researchers to use natural products as the new approaches for anti-cancer (Greenwell & Rahman, 2015).

Indonesia has the largest biodiversity in the world with at least 7,000 out of 30,000 plants recognized as medicinal plants (Paisley et al., 2017; Bermawie, 2004). Plants have bioactive compounds that are known to exert several biological effects inducing anticancer by either targeting

or activating the metabolic and signaling pathway or DNA repair gene (Iqbal et al., 2017). *Laportea decumana* or known as Daun Gatal is one of the local plants found in New Guinea that has been known for its analgesic effect especially to relieve muscle pain. Study carried out by Simaremare in 2014 and Paisey et al in 2017 regarding the phytochemical analysis of ethanolic extract *L. decumana* showed the presence of alkaloid and triterpenoid which are known to exert anti-inflammatory activity. Besides its anti-inflammatory activity, alkaloid and triterpenoid are known to exert significant anticancer effects through several mechanisms of action (Ghante & Jamkhande, 2019; Mondal et al, 2019). Nevertheless there is no or lack of research that observes *Laportea decumana* as anticancer.

In this project, the different concentrations of methanolic extract *Laportea decumana* leaf were used to treat the cell lines. Referring to previous experiments conducted by the author, the methanolic extract *Laportea decumana* shows the presence of alkaloid, terpenoid, and triterpenoid just like in ethanolic extract that was conducted by Simaremare in 2014 and Paisey et al in 2017. Ethanol and methanol were both amphiphilic polar solvents with similar polarity and the result of the phytochemical test should be quite similar and the result of ethanol extract could be used as references since the test is qualitative. The cytotoxic effect of methanolic extract *Laportea decumana* was measured towards both cancerous cell line (HeLa cell) and non-cancerous cell line (HEK cell) and the mechanism exerted of the extract to induce cell death was observed by MTS assay and DNA ladder assay, respectively. The effect of the variant treatment group of the extract on cancer cell migration was examined by scratch wound assay, and the clonogenicity of the cell following the treatment was observed by clonogenic assay.

1.2 Objectives

The objective of this research is to evaluate the cytotoxic effect induced by methanolic extract *Laportea decumana* as well as explaining the possible cell death pathway mechanisms exerted to inhibit the cancer cell growth.

1.3 Research Hypothesis

The methanolic extract *Laportea decumana* inhibit clonogenicity, migratory and, induced cytotoxic effect selectively on cancerous cell line (HeLa cell) with minimum effect on non-cancerous cell line (HEK cell) through apoptosis.

1.4 Research Scope

The scope of experiment that will be carry out in this study includes the following :

- Animal cell culture; HeLa and HEK cell culture
- Treatment of HeLa and HEK cell with different concentrations of methanolic crude extract *Laportea decumana*
- Evaluation of cell viability with implementation of MTS assay
- Detection of genomic DNA fragmentation induce by the extract as hallmark of apoptosis or necrosis by DNA Ladder assay
 - DNA Extraction and Isolation
 - DNA Gel electrophoresis
- Scratch Wound Assay
- Clonogenic Assay