CHAPTER 1: INTRODUCTION

1.1 Study Background

Among the many causes of death, cancer has ranked second as the major contributor of mortality worldwide which accounts for almost 10 million deaths all over the world, and more than 200,000 deaths in Indonesia alone (WHO, 2020). In Indonesia, highest number of new cancer-related incidence is caused by breast cancer, cervical cancer, lung, and colorectal cancer, respectively (Global Cancer Observatory, 2020). Colorectal cancer (CRC) is a malignancy arising from the colon and rectum area. CRC is on the third rank of common cancer around the world and the incidence is projected to rise in Indonesia due to the change of lifestyle including eating and exercise habits as well as the lack of regular colonoscopy screening (Mármol et al., 2017; Global Cancer Observatory, 2020; Abdullah et al., 2012).

According to Singh & Singh (2018), numerous types of treatments have been utilized to fight cancer including immunotherapy, radiation therapy, surgery, and chemotherapy. Chemotherapy targets the cells that replicate at a high rate meaning that it does not only target cancer cells, but also hair cells, GI tract mucosa, as well as bone marrow, leading to a lot of undesired adverse effects such as hair loss, nausea, and anemia (Nurgali, Jagoe, & Abalo, 2018; Ramirez et al., 2009). In clinical settings, the commonly used chemotherapy drug for CRC is 5-Fluorouracil (5-FU) which works by thymidylate synthase (TS) inhibition as well as its metabolites association to RNA and DNA of cancer cells, blocking the elongation process and leading to DNA fragmentation and apoptosis (Longley, Harkin, & Johnston, 2003). The long-term side effect of treatment with 5-FU are memory and cognitive impairment or “chemo brain” or “chemo fog” (Wigmore et al., 2010). Therefore, scientists have been researching cost-effective plant-derived natural therapies containing phytochemical compounds with cytotoxic effects while producing

less side effects (Ramirez et al., 2009; Wang et al., 2012; Greenwell & Rahman, 2015).

Indonesia is rich in biodiversity with 7.000 out of 30.000 plant species recognized as medicinal plants (Paisey et al., 2017; Bermawie, 2004). The stinging tree (*Laportea decumana*) or also called “Daun Gatal'' originated from Papua, Indonesia is known for its analgesic effect to ease muscle pain, anti-inflammatory properties, as well as antioxidant properties (Simaremare, 2018; Nandhira, 2019). Phytochemical screening for *L. decumana* leaves reveals that it is positive for alkaloids and terpenoids which are known for their anti-inflammatory effect (Simaremare, 2014; Hestiningtyas et al., 2019). Alkaloids have been used as anti-cancer drugs as it is able to trigger apoptosis and cell-cycle arrest through various pathways (Mondal et al., 2019). Terpenoids are also known for their anti-cancer activity through inhibition of Bcl-2 and NF-κB (Yang & Dou, 2010). However, there has not been much research done on investigating the anticancer properties of *L. decumana*. Hence, to check its potential to be a novel plant-derived anticancer drug, analysis on *L. decumana* extract must be done.

Referring to a previous study conducted by the author, *L. decumana* extract contains an alkaloid, terpenoid, and triterpenoid. The result of the previous study also shows that *L. decumana* extract has selective cytotoxicity on cervical cancer cell line (HeLa). In addition, *L. decumana* extract is able to inhibit the migration and colony formation of HeLa cells as well as induce DNA fragmentation and apoptosis via BCL-2 and BAX pathways. This study investigates the anticancer activity of *L. decumana* methanol extract towards the colon cancer cell line (HT29). Various concentrations of *L.decumana* extract were used for MTT assay to measure its cytotoxic effect towards HT29 cells. The effect of *L. decumana* treatment towards cancer cell migration and colony formation ability was observed by scratch assay and colony formation assay, respectively. The anticancer mechanism of action of *L. decumana* extract is observed using RT-qPCR.

1.2 Study Objective

The objectives of this study are:

- To investigate the anticancer activity of *L. decumana* methanol extract towards the HT29 cell line
- To assess the effect of *L. decumana* methanol extract on HT29 cell migration and colony forming ability
- To analyze possible cell death mechanisms exerted by the *L. decumana* methanol extract to induce a cytotoxic effect on cancer cells.

1.3 Research Scope

This study focuses on the identification of cytotoxic properties on methanol extract of *L. decumana* leaves against HT29 cells. The coverage of this study includes:

- HT29 cell culture to maintain the cell in a good condition for the subsequent assays
- Treatment of HT29 cell line with different concentrations of *L. decumana* methanol extract to observe the effect of extract in a dose-dependent manner
- MTT Assay of HT29 after treatment with *L. decumana* extract to observe the effect of extract on HT29 cell viability and determine the IC_{50} value of the extract on HT29 cells.
- Scratch Assay of HT29 cells during treatment with *L. decumana* extract to observe the effect of *L. decumana* extract towards the ability of HT29 cells to migrate
- Clonogenicity Assay of HT29 cells after treatment with *L. decumana* extract to observe the effect of *L. decumana* extract towards the ability of HT29 cells to form colonies
- DNA extraction and DNA ladder assay of HT29 after treatment with *L. decumana* extract to observe the effect of extract on DNA fragmentation of HT29 cells

- RNA extraction, cDNA synthesis and RT-qPCR of HT29 after treatment with *L. decumana* extract to observe the effect of extract on changes of BAX and BCL-2 gene expression and analyze the possible cell death mechanisms exerted by the extract.

1.4 Research Hypothesis

This study hypothesizes that the methanol extract of *Laportea decumana* is able to induce cytotoxic effect on HT29 cell line through apoptosis as well as inhibit cell migration and colony formation ability of cancer cells.