CHAPTER 1

INTRODUCTION

1.1 Project Background

Colorectal adenocarcinoma implicates as the tumor of the rectum or large bowel, including the appendix. The tumor usually arises from the colorectal mucosa, and adenocarcinoma is the most common form of colorectal cancer with more than 95% cases (Thrumurthy *et al*., 2016). It is ranked at 10th most common cancer in the world, as well as Indonesia (Sudoyo *et al*., 2010). In developing countries such as Indonesia, the incidence of colorectal cancer (CRC) increases sharply after the age of 50 years old (Sudoyo *et al.*, 2010). Not only in older age group, people age less than 40 years old are also able to suffer from CRC, known as a hereditary type colorectal cancer or hereditary non-polyposis colorectal cancer (HNPCC) (Sudoyo *et al.*, 2010). Symptoms of CRC consist of abdominal pain, change in bowel habit, rectal bleeding, and microcytic anemia. There has been a reported increase of CRC by 2012 in countries that were previously considered as low-risk regions (Spain and East Asia), this rise is correlated by high-fat diets in red and processed meat, physical inactivity, excessive alcohol consumption, and smoking (Thrumurthy *et al*., 2016). Treatments given to patients are chemotherapy, radiotherapy, and surgery, which are the gold standard for cancer treatment. Despite the fact that these gold standard treatments are able to significantly improve overall survival, these treatments require high cost with such extensive side effects and may as well decrease a patient's quality of life quality. Studies by Bhatnagar & Satija (2017) shows that complementary therapies have beneficial effects in cancer patients for symptomatic relief such as chemotherapy-induced nausea/vomiting, distress, fatigue, and pain using natural product-based therapies. Therefore, an alternative medicine using a natural product is sought through giving that it provides more affordable treatment with fewer side effects.

According to the WHO, up to 80% of developing countries rely on traditional medicine for their primary health care (WHO, 2020). Medicinal plants itself serve as nature's gift to humans to help them pursue better health (Iqbal *et al*., 2017). Specific plants have bioactive compounds from their primary or secondary metabolites that are known to have a significant role in either inhibiting cancer cells by activating proteins, enzymes and signalling pathways or by activating DNA repair mechanisms including antioxidant action (Iqbal *et al*., 2017). For instance, the activity of "*nyirih*" is regarded as a mouth-cleaning activity since the 13th century, when toothbrush and toothpaste were not common (Tjahjono, 2018). *Nyirih* is made up of areca nuts wrapped around betel leaf. It is believed that consuming betel leaf and areca nuts daily would help strengthen one's teeth and gum while preventing bleeding and inflammation. Study by Majumdar & Subramanian (2019) show that betel leaf has anticancer properties such as induction of apoptosis, cell cycle arrest, and inhibits epithelial-mesenchymal transition (EMT) in cancer cells. While another study by Ng *et al.* (2014) shows that betel leaf enhances the cytotoxic potential of 5-fluorouracil in inhibiting the growth of colon cancer using HT-29 and HT116 cell lines, referring that betel leaf causes cell death by media

ting cell cycle arrest. The study stated that the active compound of *Piper betle*, hydroxychavicol (HC) is responsible as an anti-proliferative and anticancer agent. Another study by Meutia *et al*. (2017) also shows antioxidant and cytotoxic activities of areca nuts. The antioxidant activity of areca nuts is known to play an active role in repairing DNA damage in cancer cells (Keshava *et al.,*2018). Both betel leaf and areca catechu nuts contain similar phytochemical compositions which are alkaloids, flavonoids, terpenes, saponins, and tannins (Paranjpe *et al.*, 2013).

In this project, both *Piper betle* (betel leaf) and *Areca catechu* (areca nut) are extracted and added to the cell lines media in different concentrations. The cell lines morphology and viability are then observed to analyze the cytotoxicity of the phytochemical on both adenocarcinoma cell line (HT-

29) and human embryonic kidney cell line (HEK 293). The synergistic effects from the combination of *piper betle* and *areca catechu* are also observed using the same parameter.

1.2 Research Objective

Objective of this research project is listed as below:

- To extract *Piper betle* and *Areca catechu* using maceration method
- To investigate the phytochemical compound in the extract
- To observe morphological changes in the cell lines treated with extract.
- To analyze the cell's viability using MTS assay
- To investigate synergism of both extract in inhibiting cancer cell proliferation

1.3 Research Hypothesis

The *Piper betle* and *Areca catechu* extract will successfully cause morphological changes and reduce the cell viability in colorectal adenocarcinoma cell line (HT-29) without affecting the human embryonic kidney cell line (HEK 293).

1.4 Research Scope

This study will focus on the cell culture where the reaction between the cell lines and the extraction occurs. The specific scope of work that would be done in this study include;

- Extraction of phytochemicals from *Piper betle* and *Areca catechu* using maceration method ·
- Cell line maintenance of HT29 and HEK293

- Treatment of HT29 and HEK 293 with *Piper betle* and *Areca catechu* extract
- Morphological observation of treated HT29 and HEK 293
- Evaluation of the cell line viability after induced with *Piper betle* and *Areca catechu* extract using MTS Assay