Chapter 1: Introduction

Colorectal cancer is commonly abbreviated as CRC and can be termed as either colon cancer or rectal cancer based on its origin (Milan et al., 2015). CRC develops from an adenomatous polyp following a cascade of steps. Over 76.7% of CRC cases in Indonesia are detected in Stage 3 and 13.1% in Stage 4 (Widjaja & Yo, 2016). Treatment for CRC involves the use of chemotherapeutics such as 5-Fluorouracil, which results in serious side effects such as bone marrow suppression leading to neutropenia and infections and gastrointestinal toxicities such as stomatitis, nausea, vomiting, and diarrhea (Cheung, 2008). Hence, it is imperative to develop a novel alternative for CRC treatment.

A recent study has found that statins, a common class of cholesterol-lowering drugs, may play a role in improving CRC prevention and treatment even when administered based on therapeutic doses between 0.1 µM and 0.4 µM, meant for cardiovascular diseases (Dobrzycka, M., Spychalski, P., Lachinski, A., Kobiela, P., Jedrusik, P., & Kobiela, J. 2018). Another study has found a scientific base for ethyl acetate extract of *Annona muricata* (EEAM) leaves in CRC (Moghadamtousi, S., Karimian, H., Rouhollahi, E., Paydar, M., Fadaeinasab, M., & Abdul Kadir, H. 2014).

Simvastatin is a fungal derived statin. Statins are HMG-CoA reductase inhibitors commonly used to treat hypercholesterolemia (Stancu & Sima, 2001). Statins have demonstrated cytotoxic effects in combination with ajoene, an extract of *Allium sativum,* in several *in-vitro* studies of melanoma, as well as in combination with standard chemotherapeutics. They have reported tumor cell death by inhibiting the mevalonate pathway, thereby altering the prenylation of some proteins (Ledezma, E., Witting, O., Alonso, J., & Cardier, J.E. 2009; Hindler, K., Cleeland, C., Rivera, E., & Collard, C. 2006). They have been associated with lower overall mortality and CRC-specific mortality (Cai, Zhang, Wang, Luo & Zhou, 2015).

Annona muricata has been studied extensively for its therapeutic potential to treat diseases and conditions such as fever, malaria, and gastrointestinal problems. Their leaves contain 212 bioactive compounds, and ethyl acetate extracts of their leaves have demonstrated G1 cell cycle arrest and

apoptosis induction. Further, ethyl acetate itself has a polarity index of 4.4, which is relatively low, making it an ideal solvent for extraction ("Polarity Index", 2020).

Given that both EEAM and Simvastatin alone have demonstrated notable cytotoxicity, it can prove beneficial to test the two in combination against a CRC cell line. Drug combination can also produce synergy, reduce side effects, and improve efficacy (Shenfield, G. M., 1982).

In this study, the combined effect and the individual effects of Simvastatin and EEAM were investigated through a cytotoxicity assay known as MTS assay. In these assays, the amount of MTS tetrazolium reduced by mammalian cells depicts the number of viable cells present (Riss et al., 2013). The raw data obtained will be processed and statistically analyzed.

This study was conducted using the CRC cell line HT-29 established in culture from colorectal adenocarcinoma for the MTS assays as part of the *in-vitro* analysis. (Fogh, 1977). Further, for the *in-silico* analysis, Simvastatin and cytotoxic EEAM (anonnnaine, annonamine, asimilobine, and isolaureline) were subjected to Virtual Screening (VC). VC is a useful technique in drug discovery (Lavecchia & Giovanni, 2013). VC is of two main types, Structure Based Drug Design (SBDD) and Ligand Based Drug Design (LBDD) (Surabhi & Singh, 2018). In this study, LBDD was used.

It was done by subjecting Simvastatin and cytotoxic compounds of EEAM to blind docking with β-catenin, an essential protein in CRC progression (Pino & Chung, 2010). This enabled the understanding of Simvastatin and EEAM's interaction with β-catenin and helped suggest its effects on CRC progression.

This study's broad objective was to investigate and analyze single and combinatorial treatment effects of Simvastatin and EEAM on CRC management through *in-vitro* and *in-silico* approaches. There were four specific objectives: prepare and use EEAM; establish and use HT-29 and HEK-293 in culture; conduct MTS assay to & calculate Combination Index (CI) values from the drug combination to assess for synergy, agonism, or antagonism and then statistically analyze the findings; and perform molecular docking on Simvastatin and EEAM to understand their effects on CRC progression better.