CHAPTER 1: INTRODUCTION

1.1 Introduction

Cancer remains one of the major causes of death in the world and is expected to steadily increase in incidence and mortality rates in the next few decades. Colorectal cancer, the third most commonly diagnosed cancer, affected 1.9 million people in 2020 and is predicted to increase in occurrence up to 70% by 2040 (Sung et al., 2021; Høydahl et al., 2020). Unfortunately, existing first-line therapies such as chemotherapy have been observed clinically to exhibit high toxicity and cause severe side effects (Bray et al., 2018). Furthermore, the phenomenon of chemotherapy resistance in patients may occur due to genetic mutations of malignant cells and render the drug ineffective (Riganti & Contino, 2019).

Drug repurposing is a strategy suited for the development of alternative treatment of cancer which utilizes approved or investigational drugs that were initially not intended for cancer therapy (Zhang et al., 2020). Exploiting natural products for cancer treatment is also an important field of research that often yields notable curative effects. Combination of repurposed drugs and natural compounds bears a high potential to exhibit a more potent effect compared to single-agent therapy. Despite this, identifying an ideal drug pairing may be a challenging feat as drug-drug interactions yield either beneficial, neutral or harmful effects (Sauter, 2020). Hence, it is of great interest and significance to study the combination of natural products and repurposed drugs to identify potential clinically relevant therapeutic combinations (Pemvoska, Bigenzahn & Superti-Furga, 2018).

This study investigates the combinatorial effects of simvastatin, a HMG-CoA reductase inhibitor, and the ethyl acetate extract of soursop plant (EEAM; *Annona muricata*) through in vitro and in silico approaches. Simvastatin has exhibited potential anti-tumor effects through the induction of apoptosis in multiple cancerous cell lines while soursop is well known for its wide range of ethnomedicinal properties, including anti-cancer effects. Cell viability, colony forming and wound healing assays were

conducted to determine the therapeutic effects of single drug and combinatorial treatments on HT-29 cells. Preliminary results indicate that the combination yielded in antagonistic interaction, characterized by combinatorial index (CI) values above 1 (CI>1) which is contradictory to our initial hypothesis. To investigate the mechanisms of antagonism, in vitro assays such as colony forming assay and cell migration assay will be performed. Additionally, in silico studies to identify protein-protein interactions (PPIs) and molecular docking will be conducted to establish possible competitive interactions of bioactive compounds present in EEAM and simvastatin with cell death regulatory proteins.

1.2 Objective

The study aims to:

1. Conduct in vitro analysis to evaluate the effects of simvastatin and EEAM drug combination by quantifying the cell viability, migration rate and clonogenic capacity of treated HT-29 cells.
2. Perform in silico analysis to establish potential competitive interactions of bioactive compounds present in EEAM and simvastatin with cell death regulatory proteins.