CHAPTER 1

INTRODUCTION

1.1. Background

Accumulations of factors responsible for cancer formation have contributed to the durability of cancer in human body, leading to a life-threatening condition. Cancer is responsible for 9.6 million deaths and 18.1 million new cases in 2018, also positioned as the second leading cause of global death (Bray *et al.*, 2018; WHO, 2018). Cancer is not a single disease. It appears from mutated genes in any organs and shifts a normal into an uncontrollable abnormal cell with the characteristics of selective growth and proliferative advantage, metabolic reprogramming, altered stress response, immune modulation, microenvironment alteration, angiogenesis induction, as well as invasion and metastasis (Fouad & Aanei, 2017). Cancer occurs at substantial variation across and within countries due to complex interaction between host and cancer risk factors that are linked to aging population, genetic vulnerability, socio-economic status, as well as individual lifestyle (Bray *et al.*, 2018).

With the advance of research and development, there are many options provided for cancer treatment. A patient might be subjected to a single or combination therapy of the available treatment options such as surgery, radiation, chemotherapy, targeted therapy, immunotherapy, hormone therapy, stem cell transplant, and precision medicine (National Cancer Institute, 2017). Among these options, chemotherapy is still used as the most frequent systemic therapy. It potentially affects all parts of the body through the circulatory system to block cell division. Chemotherapy remains as the best option for standard protocol and widely distributed to combat various types of cancer, especially at the late stage (Miller *et al.,* 2016). Unfortunately, the development of drug resistance has reduced the effectivity of certain chemotherapy to manage cancer. This condition, along with undesirable side effects of chemotherapy, has increased the demand to discover a novel agent with greater therapeutic efficacy and fewer side effects (Demain & Vaishnav, 2011).

Many chemotherapies that serve as effective medicine for over 50 years in the market are isolated from natural resources, mainly plants and microbes (Demain & Vaishnav, 2011). With microbes as the major sources, microbial-based chemotherapies are classified in antitumor antibiotic class as it was firstly identified as antibiotic. Bacteria are type of microbes that demonstrated growing number of studies for their role in cancer therapy. The use of bacteria for cancer treatment was initiated by William Coley (1862–1936), who utilized beta-hemolytic *Streptococcus pyogenes* and *Serratia marcescens* supernatants to create "Coley's Toxin" (Łukasiewicz & Fol, 2018). His study resulted in very promising approach, marked by complete cure in more than a quarter of hundreds patients. Since that moment, many molecules with anticancer properties have been isolated from bacterial species. Firstly isolated antitumor antibiotic, Actinomycin D, was isolated from Actinomyces bacteria (Demain & Vaishnav, 2011), with the effectivity to treat cancer in children including Ewing sarcoma, Wilms cancer, neuroblastomas and trophoblastic tumors (Karpiński & Adamczak, 2018). Further review by Karpiński & Adamczak (2018) has showed that bacteria are able to produce anticancer proteins and peptides such as bleomycin, doxorubicin, and mitomycin as another types of antitumor antibiotic with a great effectivity in several types of cancer. Most of these bacterial components are defined as complex bioactive products, also known as secondary metabolites. Secondary metabolites are pivotal components of bacteria and other natural products. They do not involve directly in normal growth and development of an organism, but have highly contributed in aiding longer lifespan since they are used to treat diseases (Demain & Vaishnav, 2011).

Secondary metabolites are the products of metabolism which might evolve in nature following the environmental condition. This leads to the fact that bacteria inhabited in different earth region might produce its own version of unique metabolites. Following this fact, Indonesia is a part of Southeast Asian countries which considered as World's top biodiversity area (Cleary & DeVantier, 2011). The unique geological history has promoted Indonesia to potentially have a rich microbial species and endemism. However, many microbial species in Indonesia are yet to be

discovered and characterized for their potency in health support. Nevertheless, during the period 2014 – 2017, a research team of Indonesia International Institute for Life Sciences (i3L), funded by USAID Agency, conducted a research project aimed to isolate microbes from different samples within Indonesia. The project was titled "Developing a bioeconomy in Indonesia: Identification of novel microorganisms and microbial enzymes from Indonesian peat land and buffaloes manures samples to improve bioconversion of oil palm residues." This research was further called as i3L-USAID project. With the overarching goal to explore a rich biodiversity of Indonesia, more than 500 bacteria, yeast, and fungi were successfully isolated and identified at least until the genus level. Among the genus discovered, some of them have been described, or have members among the group, as antibiotic, antifungal and cytotoxic compounds producers. Therefore, this situation puts Indonesia as a promising source for identification of new cytotoxic molecule for managing cancer, in which the discovery of more effective compound from bacteria might contribute to the global medicines industries.

1.2. Objectives

The objectives of this research are:

- To screen previously isolated bacteria that are associated with decomposing plant matters from Indonesian soil samples and identify those with possible production of cytotoxic molecules
- To analyze the cytotoxicity of selected bacteria extract on cancerous and non-cancerous cell lines

1.3. Scope of the Study

This research is conducted at the Laboratory of Indonesia International Institute for Life Sciences (i3L), with the scope as follows:

- Literature study to characterize, analyze, and select the bacteria for cytotoxicity assessment

- Bacteria inoculum production using both solid (agar) and liquid (broth) media
- Crude extraction of bacteria to collect their bioactive components
- Mammalian (cancerous and non-cancerous) cells preparation that includes thawing, passaging, counting, and plating
- Mammalian cells treatment using bacteria extracts
- Cytotoxicity evaluation of bacteria extract using microculture tetrazolium test (MTT)