CHAPTER 1

INTRODUCTION

1.1 Problem Background

In 2008, cancer killed 1.3 out of 10 people in the world and it is predicted that this number will be increased by 4.5 fold in 2030 (WHO, 2012). Similar projections were made in a recent study based on UK population data, the numbers suggest 42.47% increase in cancer incidence as well as 30.06% increase in cancer mortality between 2014 and 2035 (Smittenaar, Petersen, Stewart, & Moitt, 2016). Of course, these numbers might differ in developing countries as socioeconomics, demography, growth and aging rate, as well as enforcement of preventive measures are important factors in prediction models (Thun, DeLancey, Center, Jemal, & Ward, 2010). It is also important to take into account that the definition and diagnostic criteria for cancer evolve as knowledge and technology advance, and that will significantly affect the relevancy of prediction models (Hakulinen, 1996). Nevertheless, it serves as a point of reference and brings attention to medical professionals of the need for better cancer treatment in the near future.

Cancer prevalence is determined by combining the number of new cases (incidence) and existing cases. In 2013, cancer prevalence was 1.4% of Indonesia's population (about 347,792) with cervical cancer and breast cancer being the top two on the chart. Meanwhile, the highest cancer mortality was led by breast cancer, followed by cervical cancer and liver cancer (Kementrian Kesehatan RI Pusat Data dan Informasi Kesehatan, 2015). According to the 2035 projection model, cervical cancer's incidence is predicted to increase by 48.69% at exactly 4,792.35 women per 100,000 individuals; that means 1 out of 20 females we meet is likely to have cervical cancer (Smittenaar et al., 2016). As for breast cancer incidence, it is predicted to increase by 29.52%, we can conclude that cervical cancer has a more pressing urgency between the two cancers.

In general, cervical cancer mortality is influenced by factors including but not limited to: screening (Andrae et al., 2012; Darlin, Borgfeldt, Widén, & Kannisto, 2014; Rustagi et al., 2014), stage of which cancer is diagnosed (Cuschieri et al., 2014; Darlin et al., 2014) and treatment (Markman, 2014; Medlin, Kushner, & Barroilhet, 2015; Menderes, Black, Schwab, & Santin, 2016; Pesee, Kirdpon, Puapairoj, Kirdpon, & Prathnadi, 2013; Somashekhar & Ashwin, 2015). But in Indonesia, the number of professionals who can administer screening is limited, public awareness of cervical cancer is low and most diagnosis results in advanced stages of cervical cancer (Nuranna et al., 2012). There is a need for government intervention where time and manpower are managed to tackle these problems, but as academics and researches, we can help by focusing on cervical cancer treatment.

At the time of this writing, there are four treatments available for cervical cancer in Indonesia such as surgery, radiation therapy, chemo-radiation, and neoadjuvant chemotherapy (Andrijono et al., 2013). However, the cost of a single treatment (surgery and non-surgery) ranges from 8.7 million rupiah to 42.8 million rupiah (Faktakanker.com, n.d.). Another study compared the percentage of monthly drug prices to the approximate monthly salary in middle-income countries and found that anticancer drug pricing is 5 to 14 times of an average salary (Goldstein et al., 2017). These numbers may prove to be problematic for the majority of newly diagnosed cervical cancer patients as they ranged from the age group 40-64-year-old, the highest populace is in the 45-49 year age group (Bruni et al., 2017). This suggests that patients are either nearing the end of their productive age or have already retired, they would surely struggle to afford long-term treatment plans. There is also one other issue that needs to be addressed. Not only are anticancer drug prices expensive, but it is steadily increasing over the years at the rate of 18% increase (after counting inflation) from 2006 – 2012 (Gordon, Stemmer, Greenberg, & Goldstein, 2018). As we can see here, the number of cervical patient cancer is predicted to grow in huge amount and as time pass patients will be met with growing expenses as well. Therefore, there is a pressing need to find an alternative medication that is affordable and effective against cervical cancer.

Indonesia is home to a huge number of flora and fauna, with over 36 million ha of terrestrial protected area and 30,000 km2 seagrass plain, which are both diverse and endangered (Ministry of the National Development Planning, 2016). According to Indonesia's environment and forestry ministry, over a thousand coral reef locations can be found in 2013 but only about 5% have 75-100% life coral coverage (Ministry of Environment and Forestry of Indonesia, 2014). This is alarming for a number of reasons since it meant the loss of industrial and pharmacological benefits. Hundreds of publications on marine compounds were released in the past decade and the majority of them are focused on cancer medicine (Ruiz-Torres et al., 2017). Due to its harsh environment, many marine organisms produce secondary metabolites that could be used as new drugs (Malve, 2016). Marine seaweed, for example, is known for their polysaccharides which have seen much use as food ingredients, but recent findings show that they also possess anticancer and cancer preventive properties (Fedorov, Ermakova, Zvyagintseva, & Stonik, 2013). Thus it would be beneficial to cultivate marine seaweed both for the preservation of marine biodiversity as well as the development of cancer drug.

Brown algae have comparatively higher antioxidant activity than red and green algae (Machu et al., 2015), it is easy to harvest due to its abundance and relatively simple to mass produce (Buschmann et al., 2017). It shows anticancer properties against large amount of cancer types such as pancreatic cancer (Geisen et al., 2015), lung cancer (Liu, Kuang, Wu, Jin, & Sun, 2016), colorectal cancer (Park et al., 2017) and breast cancer (Moussavou et al., 2014). This is because brown algae produces compounds such as fucoidan (Isnansetyo, Lutfia, Nursid, Trijoko, & Susidarti, 2017; Kim, Park, Lee, & Park, 2010; Rodriguez-Jasso, Mussatto, Pastrana, Aguilar, & Teixeira, 2011), fucoxanthin (Ganesan, Matsubara, Sugawara, & Hirata, 2013; ROKKAKU et al., 2013) and polyphenols (Aravindan, Ramraj, Somasundaram, Herman, & Aravindan, 2015; HE, LI, RANKIN, ROJANASAKUL, & CHEN, 2015; Namvar et al., 2013a) which are known to induce apoptosis in cancer.

The anticancer effect of brown algae was also observed in cervical cancer. Sulfated polysaccharide, heterofucan, extracted from *Sargassum filipendula* was shown to induce apoptosis in HeLa cells. This is achieved by the activation of the intrinsic apoptotic pathway, confirmed by decreased expression of Bcl-2 and increased expression of (Leandro Silva Costa et al., 2011). Methanolic extract from *Dicotyota cilliolata* and *Dicotyota menstrualis* also significantly decrease cell viability in cervical cancer line SiHa (Gomes et al., 2015).

This project serves as a preliminary study on anticancer properties of Indonesian brown algae extract obtained from extraction by methanol and ethanol. First, we need to confirm the presence of cytotoxicity from our sample. That is why we chose maceration, it is simple and does not introduce heat into the system (Grosso, Valentão, Ferreres, & Andrade, 2015). In addition, varying concentration if methanol and ethanol extract were used to determine the effect of concentration of extract on cytotoxic activity towards HeLa cells. Cell viability analysis is chosen as a parameter of cytotoxicity and will be measured using MTT analysis. We chose HeLa cells to represent cervical cancer and not SiHa since HeLa is more sensitive to apoptosis-inducing compounds (XU et al., 2012; Zhao et al., 2016) compared to SiHa cells. Phytochemical screening is chosen to investigate the metabolite composition of brown algae extract due to its simplicity and cost efficiency which is fitting for preliminary study (Sasidharan, Chen, Saravanan, Sundram, & Yoga Latha, 2011).

1.2 Problem Formulation

Based on the background above, the problems can be formulated into the following research question(s):

RQ1: Does difference in extraction solvents of Indonesian brown algae impact HeLa cell viability?

RQ2: Does concentration of brown algae extracts have any effect on reducing HeLa cell viability?

RQ3: What metabolites are present in brown algae extracts?

1.3 Research Objectives

The aims of this experiment are listed as follows:

RO1: To investigate the effect of different extraction solvents of extracts on HeLa cell viability.

RO2: To investigate HeLa cell viability level of the different extracts at various concentrations.

RQ3: To conduct preliminary phytochemical screening on brown algae extracts.

1.4 Research Scope

This study focuses on the viability of HeLa cells when treated with ethanolic and methanolic extract from Indonesia brown algae at various concentration levels. The following are the scope of research:

- Extraction of brown algae by means of maceration
- The use of ethanol and methanol as maceration solvents
- Phytochemical screening testing for polysaccharide, polyphenol, terpenoid, and tannin
- 24-hour treatment of HeLa cells under controlled CO_2 levels
- MTT analysis as a means to calculate cell viability