

# CHAPTER I

## INTRODUCTION

### 1. Background

Cancer is quickly becoming one of the leading causes of death worldwide, which increases health-care costs, a number estimated to 17 million new cases of cancer every year in developing countries according to Globocan, a WHO (World Health Organization). Cancer mainly affects poor people, frequently people with more exposure to risk factors, and often have no access to affordable care, and a global misperception associates cancer only with wealthy nations and relates it to a sedentary life, tobacco, and fatty diets (López-Gómez et al., 2013).

The development of resistance to chemotherapeutic agents is, unfortunately, the common complication in the treatment of different types of cancers in the past few decades, then a tremendous variety of chemotherapeutic agents have been introduced and most of them have various side effects. Therefore to reduce the discomfort and increase the survival rate for patients receiving chemotherapy, current efforts toward anticancer drug development and research are looking forward to finding drugs with high efficacy and low side effects. Hence, bioactive compounds from natural sources are of particular interest.

Green plants especially plant-derived medicine are worldwide well known for the treatment of diseases, either due to drug resistance or side effects of synthetic drugs and are constantly been used for their biological activity as principal indigenous health. They have also played an important role as a source of effective anticancer agents, and, significantly, 60% of currently used anticancer agents are derived from natural sources including plants, marine organisms, and microorganisms (Newman et al., 2003).

Moreover, in the past few decades, notable changes in the way of using natural medicine have been notified to maintain health care also as an alternative therapy despite many challenges. Though scientific studies on the application of many traditional medicinal plants have been ventured for ensuring their efficacy and non-toxicity, they haven't yet been supported enough. Lengkuas (*Alpina galanga*) due to its function, is well known in Indonesia as one of the plants which can be utilized in the treatment of diseases. *Alpina galanga* is a plant that belongs to the Zingiberaceae family (Kuntorini, 2005). Lengkuas is one of the plants that can be used as a cancer treatment by increasing apoptosis and inhibiting proliferation. In the investigation of Zaeoung, S., et al. (2005), it stated that fresh rhizomes of *Alpina galanga*

methanol extract as an antioxidant removes free radicals and has cytotoxic activity against MCF7 (breast adenocarcinoma) and LS174T (colon adenocarcinoma) cell (Zaeoung et al., 2005)

Galangal rhizomes contain flavonoids, tannins, terpenoids, and phenylpropanoids (Chudiwal et al., 2010). Previous research highlights that 1'-acetoxy chavicol acetate extract compound from *Alpinia galanga* extract showed strong cytotoxic activity against T47D breast cancer, WiDr colon cancer, Hela cervical cancer, and Vero normal cell line with IC<sub>50</sub> values of 3.14, 7.26, and 12.49 µg/mL, respectively (Da'i et al., 2019). In addition, previous investigations showed the greater of the anticancer activity of ACA upon numerous human cancer cell line proliferation such as lung carcinoma/ A549 cell, Leukemia/K 562 cell, HeLa cell (Rusmalin, 2003). Besides, 1'-S-1'-acetoxy chavicol acetate (ACA) isolated from galangal plant induced cytotoxicity in various cancer cells, including cervical cancer in combination with miR-629 and RSU1 (Chouni & Paul, 2018). However many research and reviews on *Alpinia galanga* showed the presence of diverse phenylpropanoid bioactive compounds. Among several compounds purified, p-coumaryl diacetate, 1'-acetoxy chavicol acetate, 1'-acetoxy eugenol acetate, and trans-p-acetoxy cinnamyl alcohol were found most potent in vitro against cancer cell. Based on the previous investigation of nine analogs of 1'-S-1'-acetoxy chavicol acetate (ACA) were found selective against seven human cancer cell lines and only ACA, 1'-acetoxy eugenol acetate (AEA), and 1'-acetoxy-3,5-dimethoxy chavicol acetate (AMCA) induced apoptosis and antiproliferative activity of growth of MDA-MB-231 breast cancer cells with a half-maximal inhibitory concentration IC<sub>50</sub> value of <30.0 µM (su Ki Liew et al., 2017). It has been highlighted that the n-hexane and chloroform lengkuas rhizome extract revealed that p-coumaryl alcohol γ-O-methyl ether compound has specific cytotoxic activity against cancer cell such as SNU638 (IC<sub>50</sub> = 1.62 µg/mL) (Rangan, 2013). Also, the previous study of Ling, Zhao & al., (2012) showed that 4-acetoxy cinnamyl acetate, 1'-acetoxy chavicol acetate showed cytotoxic activity on human lung adenocarcinoma cell A549 (IC<sub>50</sub> 19.35 µmol/L) (Ling et al., 2012).

Human has commonly used microbial bio-catalysis since thousands of years ago for the bread making, dairy products, and alcoholic drinks. Scientifically, Louis (1862) put the first scientific bases for the microbial transformation applications, when he used a pure culture of *Bacterium xylinium* used to transform alcohol to acetic acid. Subsequently, several microbial transformations' experiments have been carried out, which showed that a one-step procedure might produce a remarkable product (Alfarra et al., 2012).

Biotransformation could be defined as a specific modification of a definite compound to a distinct product with structural similarity, by the use of biological catalysts including microorganisms like fungi. The biological catalyst can be an enzyme, or a whole, inactivated microorganism that contains an enzyme or several enzymes produced in it (Hegazy et al., 2015). In other words, biotransformation is defined as the enzymatic conversion of natural and chemically synthesized product, into a substance having a specifically modified structure. Throughout the biotransformation processes, drugs and chemicals are structurally modified by various enzymatic systems to form more polar substances, which can be excreted more easily than the original compounds. Problems arise when these modifications generate toxic products. Traditionally, drug metabolism studies use in vivo experiments in mice, rat or guinea pig, or chimeric mouse models with transplanted human hepatocytes. However, these models can create ethical dilemmas; and the experiments are expensive and time-consuming. Moreover, metabolites are sometimes produced in low amounts, thus hindering their identification. The use of the bioconversion process has gained importance over chemical technologies because of process controls, manipulations of microorganisms, safety, and reproducibility. While the chemical processes generally involve high temperature, high pressure, and in some cases organic solvents. Also, microorganisms are capable to produce a great variety of enzymes in a short time as a result of its natural characteristic to multiply. It is also possible to obtain and cultivate microorganisms that can survive under extreme environments such as low or high temperatures and or acidic or alkali conditions. Microbial transformation can make feasible reactions that are not likely to be carried out by traditional synthetic procedures. Numerous bioconversion processes have employed biocatalysts such as lipases, amylases, cellulases, proteases, and xylanases for commercial production of bio-products.

Previous investigations on biotransformation studies showed that biotransformation of perillyl alcohol with *Fusarium culmorum* conducted to dehydroperillic acid with a yield of 20.4 %. Perillyl alcohol was found selective to exert cytotoxicity against HepG2 cell line with an IC<sub>50</sub> value of 409.2 µg/mL and dehydroperillic acid was found to exert cytotoxic activity against A549 cell line with an IC<sub>50</sub> value of 125 µg/mL (CE et al., 2017). Besides, microbial biotransformation of many bioactive compounds such as terpenoids, alkaloids compounds (Maré et al., 2018). Biotransformation has recently evolved as an effective technique for the production of structurally diverse molecules with a wide range of biological activities. In an attempt to search for new potent anticancer agents, various microorganisms were used for the transformation of taxanes (Xu, Zhong-mei, Zhi-yong, et al., 2011). Due to the research purposes, the microorganisms firstly were chosen exploratively and randomly. secondly,

because they were easy to obtain, and they have also successfully carried out biotransformation result in previous research due to their easy manipulation, and their high reproducibility. Therefore, it is highly expected that this present research can also biotransform compounds in the ethyl acetate fraction of *A.galanga* extract.

However, Aristantika (2019) in his research stated that the cytotoxic test and antiproliferative activity of galangal extract resulted in an  $IC_{50}$  value of 53.735  $\mu\text{g} / \text{mL}$ , where the value was less potent as an anticancer (Aristantika, 2019). Therefore, to increase the anticancer activity and potency, biotransformation methods are likely an alternative way to solve this issue.

Since in the current study, the interest is in the anti-tumor properties of this traditional herb especially to ethyl acetate fraction of *A.galanga* extract and used a biotransformation process to lead to a new metabolite that could increase the  $IC_{50}$  potency which is one of the important parameters in anti-cancer studies.

Following references that have been cited above, in one hand this research goal is more interested in investigating this study using fungus as a biocatalyst agent to obtain compounds with new structures and in the other hands to determine whether the new metabolite induced by the biotransformation process has increased the potency of the cytotoxic parameter ( $IC_{50}$ ) of ethyl acetate fraction of *A.galanga* extract on T47D by evaluating cytotoxic test using the Micro-Culture Tetrazolium (MTT) assay method.

## **2. Problem of Statement**

Does the new metabolite variant of *A.galanga* extract produced by fungus biotransformation induce a higher inhibition potency on cancer cells by decreasing the IC<sub>50</sub> value of *A.galanga* extract?

## **3. Purpose and Benefit of Study**

Based on the background and formulation of the problem above the purposes and benefits of the study obtained as follows :

### **3.1 Purpose of the study**

Investigate whether, after fungus biotransformation, the new variant of *A.galanga* extract compound was produced? And has higher potency to inhibit T47D cell lines?

### **3.2 The benefit of the study**

- 3.2.1 Provide insights information on the content about fungus biotransformation and anticancer activity of *A.galanga* extract.
- 3.2.2 Add scientific data in the field of health that can be the basis for further research.
- 3.2.3 Provide reliable information sources about alternative cancer treatments for the community.