CHAPTER 1

INTRODUCTION

1.1 Problem Background

An antibacterial substance was first discovered in the 1940s (Aminov, 2010), scientists predicted that infectious disease agents could finally be conquered. However, as time goes by the therapeutic power of antibiotics summon large and often inappropriate intake of the drugs. Several factors lead to the development antibiotic resistance among bacteria which are selective pressure, mutation, gene transfer, societal pressures, improper use, inadequate diagnostics, hospital use, and agricultural use (National Institute of Allergy and Infectious Diseases, 2011). As the further matter, in the developing countries, there is limited access to healthcare service, and the limited availability of counterfeit drugs worsen the drug resistance mechanism. Thus, the issue implicates in the increased mortality and morbidity of the patients as well as increased healthcare costs. Therefore, the discovery of novel candidate of the antimicrobial agent is urgently needed.

The genus *Klebsiella* belongs to the family of Enterobacteriaceae which is listed as one of the most critical group of multidrug resistance bacteria (WHO, 2017) responsible for nosocomial infection as well as causing other diseases such as urinary tract infections (UTIs), wound infections, bacteremia, and et cetera (Qureshi, 2017). *Klebsiella oxytoca* is one of the members of the genus *Klebsiella*. *K. oxytoca* is commonly found in the human intestinal tract, mouth, and nose and considered as opportunistic organisms (Falck, 2017). Currently, *Klebsiella* sp. and *Escherichia coli* were reported to be the most common pathogenic microorganisms to develop resistance to broad-spectrum beta-lactam antibiotics via extended-spectrum beta-lactamase (ESBL) (Chakraborty, Mohsina, Zahangir Alam, Abdul Karim, & Abu Sayem, 206). *K. oxytoca* are happened to have a resistance against various antibiotics including aminoglycosides, fluoroquinolones, chloramphenicol, and trimethoprim or sulfamethoxazole (Nathisuwan, Burgess, & Lewis, 2011). Thus, there is an

increasing need to find alternatives to current antibiotics that can be used to defeat *K. oxytoca*. There are numerous conducted studies regarding the potential health benefit of brown algae extract. However, there are currently no studies regarding the usage of Indonesian brown algae extracts against *K. oxytoca*.

Well-known as a country of high biodiversity of marine organisms, Indonesia consists of about 17,500 islands with a coastline of 81,000 km^2. It has more than 11,349 types of marine biodiversity varying from coral, seagrass, mangrove, sponge, marine fish, crustacean, echinoderm, algae, mollusk, mammals, reptile, turtle, and seabird. The algae contribute to 782 types of Indonesian marine biodiversity itself. Marine and coastal area have been providing an economic significance for approximately 25% of Indonesian (Lembaga Ilmu Pengetahuan Indonesia, 2017). Despite its high amount of marine biodiversity and potential usage, there are only a few researches that are done to investigate the potential of Indonesian marine biodiversity. Unfortunately, there are just a few types of research that conducting the experiments in the specification on the algae research.

The evolution of antibacterial biological molecules along with bacteria over million years may equip them with the ability to overcome resistant strains. Particular studies on antibacterial agents from the marine environment including both of micro and macroalgae have proof that they have natural systems to tackle pathogenic bacteria and other microbes that are present in the ocean environment (Shannon & Abu-Ghannam, 2016). Recently, numerous novel compounds have isolated from marine organisms, and many of these substances have been demonstrated to have promising biological activity. In China and Japan, macroalgae have been used as food sources as well as crude drugs for the treatment of many diseases (Trease & Evan, 1985). Some studies show that secondary and some of the primary metabolites from brown algae exhibit promising and remarkable biological activities as well as providing an essential chemical compound for new drug candidates. For example, study conducted by Kang *et al.* (2012) showed that phlorotannin derived from brown algae Ecklonia cava can be used as the preventive agent for type II diabetes and a study conducted by

Rengarajan, Rajendran, Nandakumar, Balasubramanian, & Nishigaki (2013) showed that fucoxanthin derived from brown algae exhibited preventive effects towards cancer through antiobesity, antioxidant, antiproliferative, anti-angiogenic mechanisms, and et cetera.

Brown algae are abundant and potentially renewable macroalgae that are currently explored as a novel and sustainable source for both pharmaceutical and nutraceutical purposes (Barbosa, Valentão & Andrade, 2014). Brown algae are one of the most common macroalgae that are available in Indonesia. To date, there is insufficient information that has published regarding the potential benefit of algae against pathogenic microorganism (Pina-Pérez, Rivas, Martínez & Rodrigo, 2017). The antimicrobial potential of brown algae is crucially dependent on its type, the mode of extraction that is used, and the concentration of the extract. Brown algae are known for its source of bioactive compounds. Thus, the increasing efforts to use natural-based ingredients in the formulation of innovative products have given a chance for brown algae exploration.

1.2 Research Hypothesis

Marine brown algae have been proven to exhibit bioactive molecules that have been shown to have the ability to prevent the bacterial growth (bacteriostatic) as well as killing the bacteria (bactericidal). Thus, the hypothesis of this study is marine brown algae *Sargassum* spp. crude extracts of multiple extraction methods obtained from Pari Island, Indonesia will have the antibacterial properties that work against gram-negative bacteria *K. oxytoca*.

1.3 Research Objectives

Perceiving the lack of research regarding the study of the potential usage of Indonesian marine brown algae as source of bioactive compounds, the objectives of the study are:

- To investigate the antibacterial activity of crude extracts of marine brown algae *Sargassum* spp. against *Klebsiella oxytoca.*

- Compare the antibacterial activity of crude extracts of *Sargassum* spp. obtained from multiple extraction methods which are standard maceration, prolonged and agitated maceration, boiling-assisted extraction method, microwave-assisted extraction method, blending-assisted extraction method, ultrasound-assisted extraction method, and alginic acid extraction.
- To assess the inhibition of each extraction methods by performing disc-diffusion assay (Kirby Bauer method) against *K. oxytoca.*