CHAPTER 1

INTRODUCTION

1.1 Background

Salmonella is a gram-negative rod-bacteria belonging to Enterobacteriaceae family that caused a disease called salmonellosis. The genus of *Salmonella* contains two species: *Salmonella enterica* and *Salmonella bongori* (Figure 1.1). 99% of serovars belong to *Salmonella enterica* species are significant player causing human salmonellosis. *Salmonella enterica* can be further divided into six subspecies and serovars (typhoidal Salmonella and non-typhoidal Salmonella) based on their differences in the biochemical composition of carbohydate, flagellar and lipopolysaccharide structures. All *Salmonella* serotypes can be designated by an antigenic formula based on somatic (O) and flagellar (H) antigens in addition to capsular (Vi) antigens (Hurley et al., 2014).

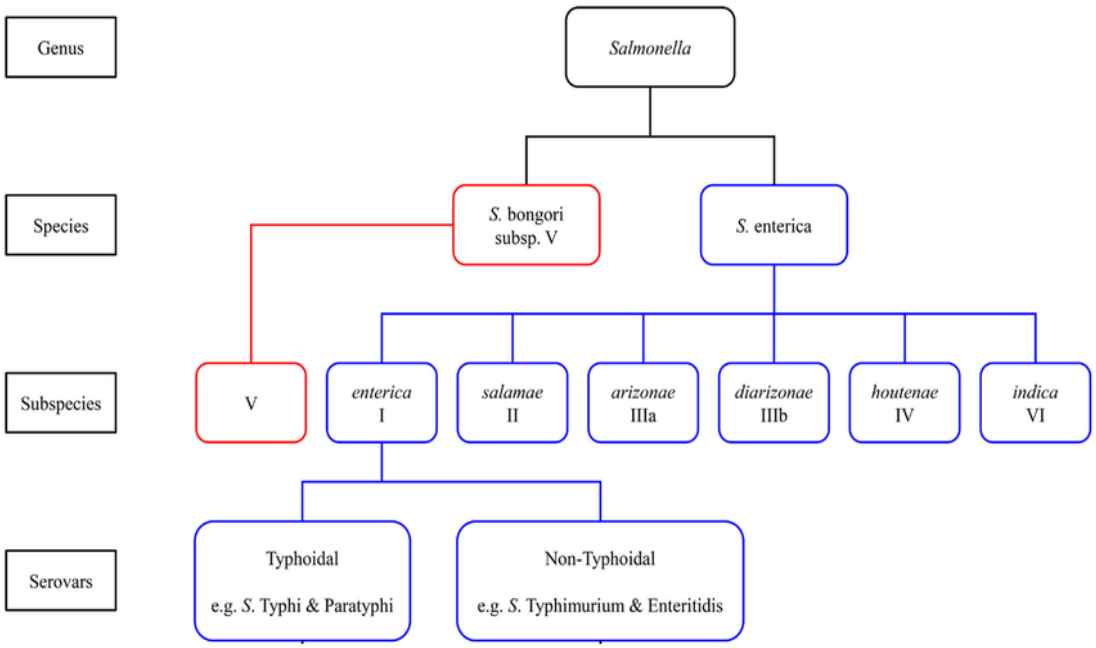

Figure 1.1 Classifications of *Salmonella* species and subspecies (Hurley et al., 2014)

Salmonella species is 1 of the 4 key global causes of diarrheal diseases worldwide. It is also one of the seven top priority bacteria which cause serious infectious problems and

resistance (WHO, 2018). This genus has been known to cause illness for over 125 years (CDC, 2018; WHO, 2018).

NTS illnesses (foodborne disease) are common in developing countries with poor hygiene and sanitation as well as developed countries and industrialized countries (Patchanee, 2008). On the global scale, non-typhoidal Salmonella caused 153 million cases of gastroenteritis and 57,000 deaths annually (CDC, 2018). In the United States alone, NTS accounts for 1.2 million illnesses and 450 deaths occur annually (CDC, 2018). Institute for Health Metrics and Evaluation Global Burden Disease 2010 Project estimated that NTS accounts for 3.4-4.8 million invasive infections and 81,300-681,000 deaths in 2010. 57% of these cases are caused by the two most reported serovar causing invasive disease: *Salmonella* serovar Typhimurium and *Salmonella* serovar Enteritidis (Crump, Sjölund-Karlsson, Gordon, & Parry, 2015). *Salmonella typhimurium* can cause infections, both in humans and animals. The important source of these bacteria comes from animal products and food or water contamination from animal feces. Moreover, the mode of transmission of this bacteria can also occur through direct contact with infected animals (farm animals are the primary reservoir) or their environment and directly between humans (Crump et al., 2015).

Worst cases of NTS can show signs and symptoms such as gastroenteritis (food poisoning), fever, abdominal pain, anemia, and malnutrition. However, in most cases, NTS produces non-specific illnesses which are difficult to recognize as infection. The infections caused by NTS are mostly self-limiting and the recovery phase of the disease usually requires no medications. However, treatment might be needed when the individual is immunocompromised or has severe complication (Sánchez-Vargas, Abu-El-Haija, & Gómez-Duarte, 2011; CDC, 2017).

The multi-drug resistant (MDR) strains of *Salmonella* spp. are immune to traditional first-line antibiotics, such as Chloramphenicol, Ampicillin, and Trimethoprim-

sulfamethoxazole (CDC, 2018). As a result, Fluoroquinolones, Ciprofloxacin, and Ceftriaxone are considered as drugs of choice for treating adults. Unfortunately, based on CDC's data (Figure 1.2), the resistance is increased. The data showed that 3% of the NTS have developed resistance towards Ciprofloxacin and Ceftriaxone; about 5% resistances towards five or more types of drugs. Furthermore, it is showing the increasing trend of drug resistance toward those antibiotics with Ceftriaxone at the highest level (Figure 1.3) (CDC, 2013). This phenomenon leads to the urgency to develop new potential source of antibiotic for treating *Salmonella typhimurium.*

	Percentage of all non-typhoidal *Salmonella**	Estimated number of illnesses per year	Estimated illnesses per 100,000 U.S. population	Estimated number of deaths per year
Ceftriaxone resistance	3%	36,000	12.0	13
Ciprofloxacin resistance or partial resistance	3%	33,000	10.9	12
Resistance to 5 or more antibiotic classes	5%	66,000	21.9	24
Any resistance pattern above	8%	100,000	34.1	38

*3-year average (2009–2011)

Figure 1.2 Drug resistance of non-typhoidal Salmonella in United States (CDC, 2013)

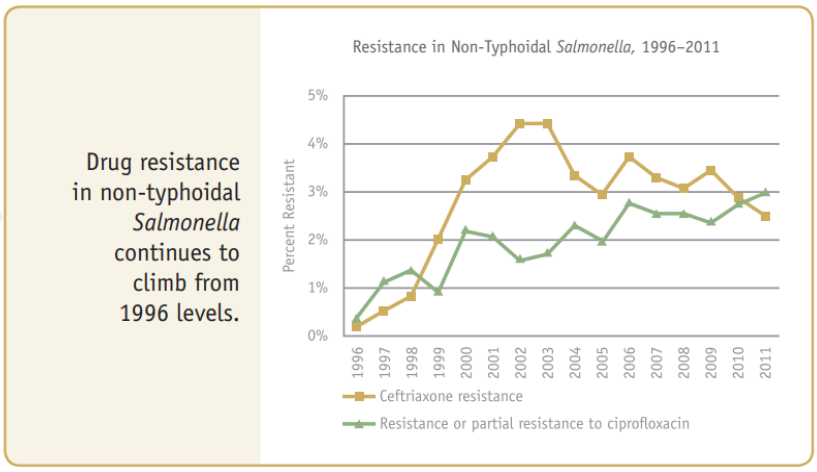

Figure 1.3 Drug resistance of non-typhoidal Salmonella from 1996-2010 (CDC, 2013)

Over the last decades, the research on the antimicrobial drug has expanded from land to the ocean to find new leads for drug candidates. The production of antimicrobial activity from marine species was noted as early as 1917 (Sridharan & Dhamotharan, 2012). The numerous compounds have been found in aquatic species with interesting

pharmaceutical activities. Approximately 3,000 active properties have been identified from 13,000 suspected marine compounds (Malve, 2016). In 2013 alone, over one thousand pharmacologically active compounds of marine origin were characterized with potential activity against cancer, viruses, bacteria, fungi, and other diseases (Malve, 2016).

Brown alga is one of the potential marine organisms which have not been fully explored but attract the attention for drug commercialization (Puspita et al., 2016). Brown algae (*Phaeophyceae*) are commonly found in the ocean or sea along continental coasts (Bajpai, 2016). There are around 1,500 species of brown alga worldwide (Encyclopedia Britannica, 2017). In Indonesia itself, there are total 111 species with eight genera of brown algae widely distributed in Indonesian tropical oceans, namely *Cystoseira* sp.*, Dictyopteris* sp.*, Dictyota* sp.*, Hormopysa* sp.*, Hydroclathrus* sp.*, Padina* sp.*, Sargassum* sp.*,* and *Turbinaria* sp. From those eight genera, it is known that *Sargassum* is the most extensively studied and scattered in Indonesia's marine (Puspita et al., 2016; DKP 2004).

The species of algae consist of a large group of biologically important marine plants in which contains essential substances for human, such as fibers, proteins, vitamins, and minerals (Rajasulochana, Dhamotharan, Krishnamoorthy, & Murugesan, 2012). Thus, seaweed or algae are mainly used as the food source and food coloring natural based. Furthermore, current studies show the potential of brown algae as the right source of antimicrobial, such as antibacterial, antifungal, antioxidant, anti-proliferative, anticancer, anticoagulant, anti-inflammatory (Pérez, Falqué, & Domínguez, 2016).

Many studies already tested the antibacterial effect of brown algae towards gram negative *Salmonella* spp. However, most of the research about antibacterial of brown algae is limited to preliminary studies, which are screening by using Kirby-Bauer method, MIC (Minimum Inhibitory Concentration), and MBC (Minimum Bactericidal Concentration). As described from studies conducted by Sridharan & Dhamotharan (2012), they used disc diffusion method to observe the comparison of the zone of inhibition between the brown

algae extracts (*Turbinaria conoides*) with the Amoxylin as positive controls. The extract show promising result as antibacterial.

The extracts to test the antibacterial effect were derived from different types of extraction. Unfortunately, the results from various studies raise concern regarding the effectiveness of extraction methods. Some studies already demonstrated that methanol extraction yields higher antibacterial activity, whereas others report that other solvent, such as chloroform or petroleum ether are better (Demirel, Yilmaz-Koz, Karabay-Yavasoglu, Ozdemir, & Sukatar, 2009; Dulgr, Hacioglu, Erduoan, & Aysel, 2009; Pandithurai, Murugesan, & Sivamurugan, 2015; Rajasulochana, Dhamotharan, Krishnamoorthy, & Murugesan, 2009; Sridharan & Dhamotharan, 2012).

The antimicrobial properties of brown algae are due to their secondary metabolites. They produce those secondary metabolites as a defense mechanism against competitor or epiphytes and also produce essential components for their growth and reproduction. These metabolites suspected to produce antimicrobial effects that inhibit or limit the competitive microorganism (Pérez, Falqué, & Domínguez, 2016). The metabolites include phlorotannins (polyphenol), fatty acids, polysaccharide (fucoidan, laminarin, and alginates), peptides, terpenes, polyacetylenes, sterols, proteins, and others compound. Furthermore, the antimicrobial activity from those compounds depends on some factors, such as the environmental conditions (light, salinity, temperature, and humidity), reproductive stage of algae, geographical location, and seasonal changes (Shannon & Abu-ghannam, 2016; Sridharan & Dhamotharan, 2012; Pérez, Falqué, & Domínguez, 2016).

Given such phenomenon, this study will test the crude extracts of *Sargassum* spp. derived from various polar and non-polar extracts, such as methanol, ethanol, distilled water, ethyl acetate, acetone, and n-hexane to look for the effective solvent as an antibacterial against gram-negative *Salmonella typhimurium*. By doing so, these findings can pave the way for further research investigation to identify the actual compounds (bioactive)

that is responsible for antimicrobial activity and can contribute to reducing the resistance of bacteria. The most important thing is to provide a starting point for the development of new antibiotic derived from the marine plant.

1.2 Hypothesis

The hypothesis is the crude extracts of brown algae from various polar and non-polar solvent exhibit antibacterial effect against *Salmonella typhimurium* based on Kirby-Bauer disc diffusion test.

1.3 Objectives

The objective of this study is first to extract the brown algae *Sargassum* spp. from various polar and non-polar solvent, and then to test the antibacterial effect against *Salmonella typhimurium* derived from those crude extracts.