CHAPTER I
INTRODUCTION

1.1 Background of the Research Study

Oceans bear a wide biodiversity of marine organisms which can serve as sources of natural products and cover about 70% of the earth (Palanisamy *et al.*, 2017). Among these organisms, macroalgae or seaweeds are great attention in marine organisms as a consequence of high demand for biodiversity in searching for functional food and medicinal drugs in many parts of the globe especially in Asian countries. Indonesia, an archipelagic country with 81,000 km long coastline, has a great potential for seaweed production: the most commonly found are red and brown seaweeds. Macroalgae, in other name, seaweeds, have variously diverse forms and sizes, including approximately 25,000-30,000 species (Santos *et al.*, 2015). Seaweeds have affluence of carbohydrate, polyunsaturated fatty acids (PUFAs) including omega-3 fatty acids, proteins, and minerals such as polyphenols, pigments (fucoxanthins, phycobilins, chlorophylls), and mycosporine-like amino acids with potential medicinal use as against cancer, inflammation, allergy, diabetes, thrombosis, obesity, lipidemia, hypertension, and other generative diseases (Canoy & Bitacura, 2018; Holdt & Kraan, 2011). Seaweeds can be classified into different nomenclature groups depending on their pigmentation, including brown seaweed (Phaeophyceae), red seaweed (Rhodophyceae), and green seaweed (Chlorophyceae) (Mohamed *et al.*, 2012).

Seaweeds grow fast and do not need cultivatable land, freshwater, or even fertilizer when compared with terrestrial plants that make seaweeds to be a demanding source for commercial applications (Lorbeer *et al.*, 2013). Seaweed or marine algae are non-flowering plants without true root stem and leaves. In the field of biological and pharmaceutical activities, the previous researchers have examined

marine algae as eccentric and attractive candidates when compared with other marine microbes and animals (Cui *et al.,* 2018). Seaweeds are one of the commercially important marine renewable resource and plenty of novel bioactive components with sulfated polysaccharide, iodine, carotenoids, dietary fibers, peptides, vitamins, proteins, minerals terpenoids, essential fatty acids, oxylipins, steroids and phlobatannins, which impact on the industry, nutritional and agricultural application (Venkatesan *et al.*, 2019).

Brown seaweed species are widely studied for their potential pharmaceutical use. One group of compounds known to be produced by brown seaweeds is polysaccharides such as laminaran, alginate, and fucoidan (Zvyagintseva *et al*., 2003). These polysaccharides are found in the cell wall matrices of the brown seaweeds and their physical and chemical characteristics are influenced by season, population age, species, and geographic location (Rioux *et al.*, 2007; Zvyagintseva *et al*., 2003). Reactive oxygen species (ROS) produced endogenously in cellular tissue such as the hydroxyl radical ($OH^{\bullet}$), superoxide radical (O_2^-), hydroperoxyl radical ($HO_2\bullet$), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$), can cause oxidative damage to DNA, protein, lipids and other cell components. They are impacted by the organism for defense mechanisms against diseases of free radicals such as anti-aging, anti-inflammatory, atherosclerosis, cardiovascular disorders, and anti-cancer (Alencar *et al.,* 2014). An antioxidant is a molecule, which prevents other molecules from oxidation. Based on the structural diversity of its secondary metabolites, Seaweeds are a wide rat of natural antioxidant (Moubayed *et al.,* 2016). The compound with antioxidant activates in seaweed include carotenoids, vitamins E, and chlorophylls, and its derivatives, polyphenol of ascorbic acid (Amorim *et al.*,

2012). Therefore, the productions of antioxidant activities from natural compounds are used for consumption because of their safety.

Diabetes is a chronic disease, which is correlated with high blood glucose level and when the body fails to utilize the insulin secretion. At present, diabetes is recognized as one of the major global health problems for all age groups. In 2017, 451 million people suffered from diabetes disease as compared to 422 million people in 2014 that is estimated to affect 693 million by 2045. Clinical and experimental research showed that diabetic is associated with serve life-threatening and damaged of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Makinde *et al.,* 2019). Alpha-glucosidase and alpha-amylase enzymes are used for the determination of diabetic activities which are one of the most effective strategies for diabetic inhibitors (Kim *et al.*, 2011). Alpha-amylase is an enzyme found in the saliva, which hydrolyzed the complex polysaccharides to oligosaccharides, which are further hydrolyzed to glucose by intestinal α-glucosidase before entering in the blood. Therefore, inhibition of these two key enzymes may assist to reduce blood glucose levels (Ademiluyi and Oboh, 2013).

Cancer is a heterogeneous disease triggered by irreversible impairment of cellular homeostasis and function. Progression of cancer cells is a result of uncontrolled cell growth and differentiation along with loss of apoptotic functions leading to a massive expansion in the neoplastic cells population (Abotaleb *et al.*, 2018). Cancer can be caused by a lack of apoptotic function, genetic mutations, oxidative stress, and hypoxia, while external causes of cancer may be linked to excessive exposure to ultraviolet rays, radiation, pollution, smoking, and stress (Abotaleb *et al.,* 2018). Cancer is the second more life-threatening disease and one of the world's most important public health problems. It can be caused in developed

and developing countries. In 2018, around 1.73 million cases of cancer and over 609,000 deaths alone were reported in the united state (Tomeh *et al.,* 2019). Mostly, crude extracts of different marine organisms have been evaluated for their anticancer activity by using *in vitro* cytotoxicity tests in malignant cell cultures. The demand for anticancer drugs is on the rise, and this requirement can be fulfilled only by strong research support. The use of cultured tissue and cells for the *in vitro* testing of cytotoxicity reduces the need for laboratory animals, and hence its popularity is on the rise (Viswanathan *et al.,* 2016). Synthetic antioxidants, antidiabetics agents to treat diabetic, and treatment for cancer disease have a variety of side effects. In order to avoid these side effects, such issues are focused by many researchers to be able to identify the potent marine compounds.

Al-enazi *et al.,* (2018) examined that the antimicrobial, antioxidant, and anticancer were extracted by using ethanol from *Laurencia catarinensis, Laurencia majuscule,* and *Padina pavonica.* According to the result, the highest antimicrobial, antioxidant and anticancer activities were obtained from Seaweed *Padina pavonica.* Fan *et al*., (2017) reported the anticancer effect of polysaccharides from *S. fusiforme* on the mice inoculated with HepG2 cells and *in vitro*. According to the results, *S. fusiforme* polysaccharide had a strong cytotoxicity effect on HepG2 cells *in vitro* and had significant inhibition of tumor growth in HepG2 inoculated mice (100-400 mg/kg bodyweight for 28 days). *Padian sp.* has demonstrated to be the main source of bioactive compounds with a wide range of pharmacological activities, such as antimicrobial, antioxidant and anticancer, antidiabetic, and cytotoxic activities (Agatonovic-kustrin *et al.,* 2017; Manivannan *et al.,* 2011; Al-enazi *et al.,* 2018; Baliano *et al.,* 2016). Isolation of different compounds from *Sargassum sp* had been reported for containing a wide range of bioactive

compounds that are sulphated polysaccharide, phenolics, plastoquinone, phlorotannins, fucoxanthin, fucoidan, sargaquinoic acid, sargachromenol, steroids, terpenoids, and flavonoids which attains a broad spectrum of biological activities that include anti-Inflammatory, antiangiogenic, gastroprotective, antibacterial, anticoagulant, antiviral, immunomodulatory, anti-diabetic, antioxidant, and anticancer activities (Vaikundamoorthy *et al.*, 2018; Narayani *et al.*, 2019; Yang *et al*., 2019; Cao *et al*., 2019; Kim *et al.*, 2018; Katherine *et al*., 2019; Milledge *et al.*, 2016). Hence, there is a need to screen more species of brown seaweed for their antioxidant, antidiabetic and anticancer activities.

In this research, the antioxidant, antidiabetic, anticancer, and toxicity with a different fraction of *Sargassum duplicatum* and *Padina tetrastromatica* from Jumiang beach, Pamekasan (without oil extraction) and Camplong beach, Kabupaten Sampang (location with oil extraction) were carried out.

1.2 The Research Problem

Based on the description in the background above, the problem that will be resolved in this study are:

1. "As medicinal algae", dose *Sargassum duplicatum* and *Padina tetrastromatica* from Madura have total phenolic content, antioxidant, antidiabetic, anticancer ,and toxicity?
2. Is there any difference in total phenolic content of *Sargassum duplicatum* and *Padina tetrastromatica* obtained from the seashore with oil extraction and non-oil extraction site at Madura Island?
3. Is there any difference in antioxidant activities of *S. duplicatum* and *Padina tetrastromatica* obtained from oil extraction and non-oil extraction site at Madura Island?

4. Is there any difference in anticancer activities of *S. duplicatum* and *Padina tetrastromatica* obtained from oil extraction and non-oil extraction site at Madura Island?
5. Is there any difference in antidiabetic activities of *S. duplicatum* and *Padina tetrastromatica* obtained from oil extraction and non-oil extraction site at Madura Island?
6. Is there any difference in toxicities of *S. duplicatum* and *Padina tetrastromatica* obtained from oil extraction and non-oil extraction site at Madura Island?

1.3 Objective of the Study

1. To evaluate total phenolic content, antioxidant, antidiabetic, anticancer, and toxicity of *Sargassum duplicatum* and *Padina tetrastromatica* from Madura Island.
2. To compare the total phenolic content of *Sargassum duplicatum* and *Padina tetrastromatica* from oil extraction and non-oil extraction site at Madura Island**.**
3. To compare the antioxidant activities of *Sargassum duplicatum* and *Padina tetrastromatica* from oil extraction and non-oil extraction site at Madura Island**.**
4. To compare the antidiabetic activities of *Sargassum duplicatum* and *Padina tetrastromatica* from oil extraction and non-oil extraction site at Madura Island**.**
5. To compare the anticancer activities of *Sargassum duplicatum* and *Padina tetrastromatica* from oil extraction and non-oil extraction site at Madura Island**.**

6. To compare the toxicities of *Sargassum duplicatum* and *Padina tetrastromatica* from oil extraction and non-oil extraction site at Madura Island**.**

1.4 Benefit of the Study

1. The research will reveal the difference in Antioxidant, antidiabetic anticancer, and toxicity of *Sargassum duplicatum* and *Padina tetrastromatica* obtained from Madura Island.
2. The research of provide information on antioxidant, antidiabetic, anticancer, and toxicity of *Sargassum duplicatum* and *Padina tetrastromatica* from an oil extraction and non-oil extraction site at Madura Island.
3. The macroalgae from Madura Island with highest antioxidant, antidiabetic, and anticancer activities will be recommended for antioxidant, antidiabetic, and anticancer drug candidate.