CHAPTER 1. INTRODUCTION

1.0. Background

Influenza, also known as the flu, is an infection caused by members of the *Orthomyxoviridae* family; influenza A, B, and C viruses. These viruses infect the airway epithelial cells, primarily that of the upper respiratory tract, due to the high expression of α2,6-linked sialic acid receptors (Denney & Ho, 2018). Influenza A and B viruses are generally responsible for causing flu outbreaks, whereas influenza C viruses only cause sporadic mild upper respiratory illness. Although most cases of influenza are self-limiting, involving clinical manifestations such as fever, sore throat, cough, headache, and fatigue, infection by certain strains of IAV can result in acute respiratory distress syndrome (ARDS) and multiple organ failure in rare and severe cases (Korteweg & Gu, 2010; Moghadami, 2017).

Not only are IAVs lethal, they also have a tendency to undergo genetic variations. Their segmented genomes give them an evolutionary advantage in the regards that they can undergo antigenic shift. This involves the co-infection of a host cell with two or more viruses, enabling the newly produced virions to attain genes from either parental strains, a process known as genetic reassortment (Chou et al., 2012; Khanna, Kumar, Gupta & Kumar, 2012). Furthermore, IAVs are also prone to antigenic drift, in which the lack of proofreading and repair mechanism allow mutations, particularly in the hemagglutinin (HA) and neuraminidase (NA), to continuously occur and accumulate, giving rise to numerous subtypes, which are categorized based on the variations of these glycoproteins (Barbezange et al., 2018; Jang & Bae, 2018; Pauly, Lyons, Fitzsimmons & Lauring, 2017). Most of these subtypes only circulate among the natural reservoir, aquatic birds, and show no tropism humans (Pulit-Penaloza, Belser, Tumpey, & Maines, 2019; Webster & Govorkova, 2014). However, if these viruses undergo mutation and jump the species barrier, novel strains of which the human population has little or no preexisting immunity could be generated, resulting in epidemics and pandemics (Perez & Garcia-Sastre, 2013; Webster & Govorkova, 2014).

One of the most well-known IAV strain that remains to be a major public health risk is the H5N1 virus, dubbed as a highly pathogenic avian influenza (HPAI). The term was coined back in 1981 to categorize highly virulent strains of avian influenza, particularly those of the H5 and H7 subtypes, which could cause 100% mortality within a susceptible poultry species (Rebel et al., 2011). Although most of these strains are restricted to poultry, H5N1 has jumped across the species barrier to mammalian hosts and caused high morbidity and mortality rates spanning throughout various continents, making it a significant public health threat (Perez & Garcia-Sastre, 2013). For example, the mortality rate of H5N1 has reached as high as 83% in Indonesia. Unfortunately, Indonesia has the second-highest cumulative number of human H5N1 cases globally, and H5N1 is endemic amongst the bird population (Adisasmito et al., 2019). In 2011, influenza's economic burden was estimated to reach as high as Rp.540,295,198,000 for hospitalized patients and Rp.831,441,508,113 for outpatients (Kosen, 2012). However, the surveillance system in this developing country is still rudimentary, and the disease burden of influenza may still be underestimated (Kosasih et al., 2013).

Getting an annual flu shot is one of the preventative measures against influenza. Due to the virus' high rates of mutation and genetic reassortment, researchers have to continuously predict the strains that may predominate in the upcoming flu seasons every year before developing an updated vaccine. The prediction is based on the probability that a currently circulating strain has high fitness and will proliferate. Nonetheless, since a predictive method is applied, the emerging antigenic variants cannot be forecasted precisely and the vaccine does not guarantee 100% protection (Morris et al., 2018; Wu et al., 2015). As for treatments, antiviral drugs are available for influenza A infections. For now, two classes of influenza antiviral drugs - matrix 2 (M2) protein inhibitors and NA inhibitors - are licensed and approved. The former category includes two drugs, amantadine and rimantadine, which have a narrower drug spectrum compared to the NA inhibitors because they target the M2 ion channel, which is unique to IAV. By blocking the M2 protein, these drugs prevent viral uncoating. However, because of widespread resistance, M2 inhibitors have been virtually abandoned and replaced by the NA inhibitors. Four drugs are included in this class - zanamivir, oseltamivir, peramivir,

and laninamivir octanoate - all of which inhibit the NA glycoprotein, preventing viral release. Although uncommon, cases of resistances against these drugs have previously been reported (Duwe, 2017; Hedlund, Larson & Fang, 2010; Huang et al., 2014; Lee & Hurt, 2018). Moreover, these medications may cause side effects and are not cost-friendly for certain demographics; among the available options, zanamivir retails the cheapest, at approximately $70 for a 5-day course, whereas peramivir costs up to $950 for a 1-day course (Ison, 2017; Schwarzinger, Lacombe & Carrat, 2003). Together, these issues pose a significant problem for Indonesian citizens and the healthcare system.

Even though the currently available prophylactic and therapeutic regimens may seem sufficient to keep the death toll of influenza at bay, the imminent threat of rapid mutations and drug resistances may jeopardize the public health should there be a sudden emergence of highly transmissible and lethal strain of IAV. Therefore, it is urgent to continuously develop novel anti-influenza agents that are effective, economical, and minimally toxic. One such potential agent comes from algal sources. Algae comprise 20,000 identified species with complex and ever-changing classification systems (Kim, Lee, Kim & Kang, 2018). These organisms have been a part of human diets for centuries and have been widely implemented in the food, cosmetics, and biomedical industry (Kim et al., 2018; Wells et al., 2017). The most common type of macroalgae consumed by humans is the brown algae, which are prolific sources of the polysaccharide fucoidan (Lorenzo et al., 2017). Responsible for the structural integrity of brown algae, they are mainly made up of sulfate and fucose, but also contain monosaccharides such as glucose and galactose. Fucoidans themselves are structurally diverse, depending on factors such as season, geographical area, and salinity. The variations in structure and content affect the pharmacological activities of fucoidan, which ranges from anti-inflammatory to anti-oxidative (Afonso, Catarino, Silva, & Cardoso, 2019; Salehi et al., 2019). Despite its well-established health benefits, the anti-IAV properties of fucoidan have not undergone extensive research. Within the past decade, only four studies tested fucoidan from three brown algae species - *Kjelmaniella crassifolia*, *Undaria pinnatifida*, and *Laminaria japonica* - against various IAVs (Hayashi, Lee, Nakano, & Hayashi, 2013; Sun et al., 2018; Synytsya et al., 2014; Wang et al., 2017). All

studies reported the effectiveness of fucoidan in suppressing the growth of IAVs. However, brown algae itself encompasses more than a thousand species and studies on three species alone cannot represent the efficacy of fucoidan from brown algae as a whole. Among the Phaeophyceae, the genus *Turbinaria* can be commonly found in tropical and subtropical waters, including Indonesia (Rohfritsch, Payri, Stiger, & Bonhomme, 2007; Wouthuyzen, Herandarudewi, & Komatsu, 2016). Yet, no anti-IAV studies have been done on fucoidans from *Turbinaria* spp.. Hence, this study is emphasized on isolating fucoidan from this genus of Phaeophyceae to provide further corroboration on the utilization of brown algae as a minimally toxic, cost-friendly treatment against IAV.

1.1. Objectives

This study initially aims to isolate and characterize fucoidan from *Turbinaria* spp. to evaluate its anti-IAV activity against IAV from clinical swab samples. Preliminary findings from the wet lab will be compared to existing literatures by conducting a systematic review, which serves to provide insights into the antiviral properties of other types of algae to support the findings of the experiments.

1.2. Hypothesis

This study hypothesizes that fucoidan from *Turbinaria* spp. is minimally toxic and can hinder IAV infection *in vitro*.