CHAPTER 1

INTRODUCTION

1.1 Background

Hypersensitivity, defined by the allergy community, is clinical manifestations that cause reproducible symptoms, initiated by the exposure to a specific stimulus at tolerable dose in normal subjects (Johansson *et al.*, 2004). According to how the immune system reacts, hypersensitivity is subdivided into hypersensitivity which involve the immunological mechanisms and hypersensitivity in which immunological reaction is excluded (Tanno *et al.*, 2016). Hypersensitivity in which initiated by the immunological mechanisms can be further subdivided into: type I hypersensitivity, known as immunoglobulin E (IgE) mediated; and type II, III, and IV, which are known as the non-IgE mediated hypersensitivity (McConnell, 2007; Uzzaman & Cho, 2012).

As reported in 2018, type I hypersensitivity was responsible for a significant worldwide morbidity and affecting almost 30% of total population (Sánchez-Borges *et al.*, 2018). Type I hypersensitivity covers atopic diseases (i.e. allergic: rhinitis, asthma, dermatitis, and conjunctivitis) and allergic diseases, including anaphylaxis, angioedema, urticaria, drug, and food allergy (Abbas *et al.*, 2020). Among those, the World Allergy Organization (WAO) reported that allergic rhinitis has the highest prevalence compared to other diseases which were included in the type I hypersensitivity (Pawankar *et al.*, 2011).

Allergic rhinitis (AR) is defined as an inflammation of the membrane lining in the nasal cavity which is indicated by one or more presence of symptoms such as sneezing, nasal itching, nasal congestion, and nasal discharge (Bousquet *et al.*, 2008). As reported by WAO, AR affected 10-30% of the total population and up to 40% of children worldwide (Pawankar *et al.*, 2011). In the Asia Pacific, AR was mostly affecting children aged 6 to 14-year-old with prevalence ranging from 3.6 -24.2% (Asher *et al.*, 2006). Meanwhile, in Indonesia, International Study of Asthma and Allergies in Childhood (ISAAC) reported that the prevalence of AR was below 5% of total Indonesian (Beasly *et al.*, 1998).

However, current evidence showed that the prevalence is gradually increasing in the big cities of Indonesia. A study by Fauzi *et al.* (2015) reported 38.2% of an overall 207 university students in Bandung were diagnosed with AR, and 66% of the cases were identified to affect the female students. Meanwhile, another report from Surabaya showed that AR affected 23% of 499 subjects from children to young adults (Soegiarto *et al.*, 2019).

The mechanism of reaction of AR is initiated when the allergen from the outside is first sampled by antigen-presenting cells (APCs) in the body (King, 2007). The APCs then present part of the allergen to the T_H2 cells through major histocompatibility complexes class II (MHC-II). The T_H2 cells then secrete inflammatory cytokines, particularly interleukin 4 (IL-4) and 13 (IL-13), triggering immunoglobulin class-switch recombination of the B cells to able to produce antigen-specific immunoglobulin E (IgE; Galli *et al.*, 2008; Uzzaman & Cho, 2012; Warrington *et al.*, 2011). Antigen-specific IgE cross-links with high affinity IgE receptors on the surface of the basophil cells, known as FcεRI receptors. When re-exposure happens, the allergen sampled by the APCs is directly presented to the antigen-specific IgE on the FcεRI receptors, leading to the degranulation of the basophil cells and secretion of inflammatory cytokines, histamine, and β-hexosaminidase (Warrington *et al.*, 2011).

Averrhoa bilimbi Linn. is Southeast Asian endemic plant species, which is currently underutilized. Previously, both the leaf and fruit of *A. bilimbi* were commonly used as food ingredients. Besides, the fruit is also used as an ethnomedicine as a skin care to remove acne and pimple (Ong & Nordiana, 1999), treat syphilis (Samuel *et al.*, 2010), whooping cough, obesity, hypertension, and diabetes (Alhassan & Ahmed, 2016). Recently, scientific studies have found that *A. bilimbi* fruit (AF), besides treating hypertension (Lestari *et al.*, 2018), hyperlipidemic (Ambili *et al.*, 2009; John & Pta, 2019), and diabetes (Kurup & Mini, 2014), has potential as antimicrobial (Mokhtar & Aziz, 2016; Norhana *et al.*, 2009), anti-inflammatory (Suluvoy *et al.*, 2017), and anticancer (Nair *et al.*, 2016). However, a study regarding the potential of AF as anti-allergy has not been conducted yet.

Phytochemical studies showed that AF is another promising source of polyphenols, including phenolic acids, flavonoids, and tannins (Hasanuzzaman *et al.*, 2013; Yan *et al.*, 2011). Hasanuzzaman

et al. (2011) was also reported that the polyphenols are also present in aqueous extract. In relation to allergies, several studies have reported the benefit of polyphenol, especially the flavonoids, as anti-allergy (Singh *et al.*, 2011; Tanaka & Takahashi, 2013). flavonoids are considered to have anti-allergy effects by inhibiting the release of inflammatory cytokines, including IL-4 and IL-13, the expression of T_H2 cells induced by the activation of FcεRI receptor-expressing cells, such as basophils and mast cells, and the expression of MHC-II on dendritic cells.

To analyze anti-allergy potential, Rat Basophilic Leukemia (RBL)-2H3 cells are frequently used for *in vitro* studies since it mimics the properties of mast cells and express high levels of FcεRI on the surface of the cells during the activation by IgE-allergen complex (Fu *et al.*, 2019). Thus, herein the potential of AF as anti-allergy was examined via *in vitro* using RBL-2H3 cells.

1.2 Objective

To analyze the anti-allergic effect of *Averrhoa bilimbi* Linn. fruit water extract (AFWE) on RBL-2H3 cells.

1.3 Hypothesis

The AFWE inhibits the degranulation process marked by declination of β-hexosaminidase release through reduction of intracellular calcium concentration of RBL-2H3 cells.

1.4 Scope of Work

The experiment was conducted to investigate the anti-allergic properties of AFWE towards inhibition of degranulation in RBL-2H3 cells. RBL-2H3 cells were cultured in 5% FBS-DMEM and sensitized with DNP-IgE. The degranulation was induced by addition of DNP-HSA, and degranulation rate was analyzed by measuring β-hexosaminidase release. Intracellular calcium concentration was also measured.