CHAPTER I

RESEARCH BACKGROUND

1.1 Introduction

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disorder characterized by pruritic, chronic eczematous skin lesions on dry skin which mostly begins in childhood, continuing in adults with increasing prevalence over the past decade (Sullivan & Silverberg, 2017). AD in children occurred 65% before patients reached the age of 18 months. Although some AD cases reach resolution by age 12 years, the report found that 60% of AD cases in childhood still persist to adulthood. In Indonesia, the prevalence of AD reached up to 24%, hence making AD one of the common skin diseases in children (Wicaksana et al., 2017). AD is considered as a multifactorial disease which involves complex interaction of environmental and genetic factors. The pathogenesis of AD was firstly attributed to an imbalance in T helper (Th)-type 1 (Th2) and Th2 response to newer immunologic pathways and skin barrier-based abnormalities. Studies also suggested that elevation of immunoglobulin E (IgE) may play an important role, similar to allergic rhinitis that are commonly present in AD patients (Kabashima, 2013). Furthermore, research has reported the major contribution of skin barrier impairment, additional inflammatory cell types, and also wider proinflammatory cytokine profiles to the pathogenesis of AD (Silverberg & Silverberg, 2015; Sullivan & Silverberg, 2017). Due to the flares, itchiness, stinging sensation, redness, and scarring which caused major impairment in quality of life, treatments are important to accelerate healing by reducing factors which worsen AD condition. Currently, the most AD treatments available are only to minimize the symptoms of itching and prevent the rash to worsen such as topical corticosteroid and moisturizing cream. Another option is utilization of anti-IL4 therapy dupilumab. However, it cannot be denied that dupilumab is very expensive and may not be accessible to many people, not to mention the appearence of many undesirable adverse effects (Tameez Ud Din et al.,

2020). Thus, many current researchers start to proceed with alternative medication through natural resources.

Looking at the opportunities, Indonesia is one of the countries in the world with high potential to produce alternative natural medicine with rich diverse natural resources. Unfortunately, despite its natural sources' richness, many endemic Indonesian plants, herbs, and spices are underutilized; its health benefit potential is understudied.

Calophyllum inophyllum or locally known as nyamplung is one of the endemic fruit bearing trees that can be found in tropical regions such as Indonesia. It has been traditionally used as herbal medicine for treatment of sore throat, burns, pain, and inflammation. The flavonoids, coumarins, triterpenoids, and xanthones are the major chemical constituents of tamanu oil which was previously reported to possess diverse pharmacological properties including anti-microbial, wound healing, antioxidant, and anti-inflammation activities (Van Thanh et al., 2019). However, there is limited study in regards to the utilization of tamanu oil for anti-inflammation treatment of atopic dermatitis. Therefore, in this experiment, the anti-inflammatory effect of *Calophyllum inophyllum* ethanol, methanol, and n-hexane seed extract for atopic dermatitis in cultured human keratinocyte (HaCaT) cells is going to be assessed.

1.2 Objective

To investigate the anti-inflammatory effect of *Calophyllum inophyllum* seed extract through in-vitro assay in HaCaT cells

1.3. Hypothesis

- The *Calophyllum inophyllum* seed extract exhibits anti-inflammatory effect in LPS-induced HaCaT cells through suppression of pro-inflammatory genes.

- *Calophyllum inophyllum* seed extract is capable of protecting HaCaT cells from cell death induced by LPS

1.4. Scope of Work

The experiment will be conducted to investigate the anti-inflammatory effect of *Calophyllum inophyllum* ethanol, methanol, and n-hexane seed extract in HaCaT cells. HaCaT cells will be cultured in DMEM supplemented with 10% FBS. The cytotoxicity of each seed extract will be analysed using MTT assay and measured using microplate reader or spectrophotometry. The effect of LPS stimulation on HaCaT cells will also be analyzed to determine the most optimum concentration, able to reduce cell viability to at least 75%. Furthermore, the protective effect of the seed extract will be evaluated on LPS-induced HaCaT cells, followed by analyzing the cell viability using microplate reader or spectrophotometry. In regards to quantitative measurement, inflammation will be induced using LPS whereas related gene regulation for inflammatory induction (including IL-1α, IL-1β, IL-6, IL-8, TNF-α, TSLP, and COX-2) is going to be assessed with GAPDH, β-actin, and RPL13A as reference gene using qRT PCR.