Chapter 1

Introduction

1.1. Background

Atopic dermatitis (AD) is an inflammatory skin disorder, the most common form of eczema, and affects at least 230 million individuals worldwide (Kim et al., 2019; Tsai et al., 2019;). Disease manifestations usually start in infancy between ages three and six, though it can occur at any age, and AD patients typically experience acute flares of pruritic lesions, eventually leading to lichenification (Huang et al., 2017; Thomsen, 2014). AD is a heterogeneous condition, with many different risk factors, such as genetic predisposition, immune dysregulation, and skin barrier disruption due to environmental insults to the skin (Al-Shobaili et al., 2016; Kim et al., 2019). However, the exact etiology is currently unknown.

The many risk factors of AD also interplay with regards to pathogenesis. In a review by Yang et al. (2020), the mechanism of immune system dysfunction is elucidated. With regard to immune dysregulation, defects in the innate immune system can contribute, with defects in cell mediated epidermal barrier repair process resulting in inflammation and leaky junctions between cells. Meanwhile, dysfunction in the adaptive immune system involves the overexpression of T-helper 2 (Th2) cytokines and immunoglobulin E (IgE) in response to antigens. As for an impaired skin barrier, a decrease in proteins with skin barrier repair properties such as filaggrin, ceramide, antimicrobial peptides (AMP), and serine protease due, possibly due to genetic mutations, as well as an increase of serine protease and tight junction (TJ) disorder has been postulated to be the cause of AD (Kim et al., 2019). In addition to this, cornified cells of the dermis make up the cornified envelope, an immensely rigid submembranous structure that protects the skin from environmental insults (Furue, 2020). Major skin barrier genes

implicated in AD include: *FLG*, which encodes for filaggrin, and *IVL*, which encodes for involucrin, a protein that contributes to the formation of the cornified envelope. Immune dysfunction-related genes are *CCL22*, *TARC*, *MDC* and *CTACK* which are genes that encode for crucial chemokines, and *TSLP*, *IL-33*, *IL-25* which encode for pivotal cytokines in AD development (Chieosilapatham et al., 2021).

Common medication for AD include topical corticosteroids, antihistamines, and topical antiseptics, with the goal of reducing the degree and number of exacerbations, as there is currently no cure (Thomsen, 2014). However, there are undesirable side effects and some treatments only treat symptoms but have no effect on the activity of the disease itself. For example, prolonged use of corticosteroids may lead to atrophy, stretch marks, and rosacea, and antihistamines only function to relieve itchiness (Coondoo et al., 2014; Thomsen, 2014). Therefore, finding novel treatments, particularly natural therapies to treat AD, is imperative in order to develop treatments that are effective towards disease activity with little to no side effects.

Tamanu oil is the natural oil obtained from *Calophyllum inophyllum,* a tree native to Africa, Maritime Southeast Asia, and Polynesia (Pribowo et al., 2021). Studies have supported that tamanu oil possess anti-inflammatory properties, antimicrobial activities, antioxidant & anti-UV properties, wound-healing activities, and dermal and epidermal extra-cellular matrix effects, and is recommended to treat skin diseases like AD, acne, psoriasis, burns, and dermatoses (Raharivelomanana et al., 2018; Dweck & Meadows, 2002).

Thus, the proposed study will investigate the activity of Tamanu oil on AD-induced human immortalized keratinocytes (HaCaT) as a treatment for AD with respect to its pathogenesis involving immune system

dysregulation by analyzing gene expression of *IL-33*, *IL-25*, *TSLP*, *TARC*, *MDC*, AND *CTACK* which are upregulated in the skin of AD patients, FLG, and IVL, which are downregulated.

1.2. Objectives

The aim of the study is to investigate the effects of the ethanol-immicible extract (EIE) of *Calophyllum inophyllum* by analyzing gene expression levels of *IL-33*, *IL-25*, *TSLP*, *TARC*, *MDC*, *CTACK*, which are known to be upregulated, and *FLG*, and *IVL*, which are known to be downregulated in TNF-α and IFN-γ-induced HaCaT, used as an AD model (Kim et al., 2018; Cha et al., 2019; Wang et al., 2022). The following objective of the study is:

- To investigate the effect of *Calophyllum inophyllum* EIE on gene expression levels of AD-related genes *IL-33*, *IL-25*, *TSLP*, *TARC*, *MDC*, *CTACK*, *FLG*, and *IVL* in TNF-α and IFN-γ-induced HaCaT

1.3. Hypothesis

- *Calophyllum inophyllum* ethanol-immicible extract will upregulate gene expression of FLG and IVL, and downregulate gene expression of *IL-33*, *IL-25*, *TSLP*, *TARC*, *MDC*, and *CTACK* in HaCaT cells that have been treated with TNF-α and IFN-γ.