CHAPTER I

INTRODUCTION

1.1. Background

Chronic inflammatory skin disorder such as Atopic Dermatitis (AD) and Psoriatic Dermatitis (PD) which significantly reduces the quality of life including disruption of daily activities, and sleep disturbance of the affected individuals. The prevalence of atopic dermatitis in adults is 7% and in children is 18% (Wang et al., 2020). On the other hand, the prevalence of psoriatic dermatitis ranges from 2% - 8.5% in adults depending on different regions. Psoriasis' prevalence is higher in females ≤18 years (Griffiths, van de Kerkhof & Czarnecka-Operacz, 2017). Individuals suffering from atopic and psoriatic dermatitis are at a higher risk of suffering infectious complication including bacteremia, eczema herpeticum (EH), endocarditis, Infectious complications of AD include asthma, allergic rhinitis, skin and soft tissue infections (SSTI), eczema herpeticum (EH), metabolic syndrome, changes in the renin-angiotensin-aldosterone system, increased risk of cardiovascular disease, osteomyelitis, septic arthritis, and endocarditis (Nutten, S., 2015; Wang et al., 2020, Carvalho et al., 2016). Atopic Dermatitis causes the skin barrier of the person to be disrupted, allowing the skin to be vulnerable towards skin allergens and irritants, triggering a Th2 immune response. In Psoriatic Dermatitis, the disease causes an uncontrolled proliferation of the keratinocyte cells accompanied with dysfunction differentiation due to disturbances in both the innate and adaptive cutaneous immune system. In both diseases, the patient suffers from itchy, dry skin or scaly plaques, as well as an increased risk of systemic complications, as mentioned previously (Rendon & Schäkel, 2019).

One of the pro-inflammatory cytokines produced by the epidermal keratinocytes, which is also produced by other immune cells is TNF-α. The TNF-α proinflammatory cytokine plays a role in thymic stromal lymphopoietin (TSLP) expression. The keratinocytes, which

makes up the majority of the skin's epidermis typically produces an increased level of TSLP, which consequently leads to the activation of the innate lymphoid cells 2 (ILC2), increasing the chances of skin infection in AD patients (Brunner, Guttman-Yassky & Leung., 2017; Wang et al., 2020), as well as induce differentiation of Th1/Th17 cells which contributes to keratinocyte proliferation and inflammatory response in psoriasis (Suwarsa et al., 2019) . This indicates that TNF-α plays a significant role in initiating Th2-type allergic inflammation. TNF-α is also capable of inducing expression of other inflammatory cytokines including IL-1α, IL-6, IL-8 and IL-33, contributing to the development of skin inflammation (Mizuno et al., 2015). Hence, HaCaT cells are used in this study with TNF-α as the inflammatory inducer to model the inflammatory response of keratinocytes.

At present, there still remains a limited option for inflammatory skin disease treatment in Indonesia. The most common treatment prescription for those who suffer from atopic dermatitis in Indonesia often involves topical medication and oral corticosteroid which functions to help alleviate the symptoms at best. Topical medications are the most common way to treat the rashes and lesions caused by Atopic Dermatitis. These topical medications include corticosteroid creams, enzyme phosphodiesterase 4 PDE4 inhibitors and calcineurin inhibitors which aids in reducing inflammation and relief itching to allow the skin to start healing (Kapur, Watson & Carr, 2018). On the other hand, psoriasis treatment is heavily determined by its disease severity, comorbidity, and patient's access towards healthcare. In mild to moderate psoriasis cases, treatment combination consisting of glucocorticoids, vitamin D analogues and phototherapy is given to patients (Rendon & Schäkel, 2019). However, these topical medication are not suited for long-term usage due to several risk factors such as stretch marks, skin atrophy, skin discoloration, skin peeling and other hormonal changes, which is not suitable for long-term usage especially in children, as it can lead to other systemic complications. Alternative medications include UV phototherapy and dupilumab treatment. Unfortunately, UV phototherapy is very time consuming, and is not considered to be feasible

for the majority of AD patients (Brunner, Guttman-Yassky & Leung., 2017) whereas the rest of alternative treatments available is still considered quite costly in Indonesia, which are not easily accessible to the public. Treatment option for psoriasis is dependent on the presence of comorbidities which affects treatment selection and the biologic drug given to each patient (Rendon & Schäkel, 2019). This demonstrates that there is still a gap in inflammatory skin disease treatment in Indonesia which is both safe and effective in the long run. For this reason, an urgent need for a natural alternative treatment that is capable of acting as a potent anti-inflammatory agent is needed as a possible treatment option for both PD and AD.

Among the numerous plants capable of inducing anti-inflammatory response, *Calophyllum inophyllum* is a promising candidate for AD treatment. *Calophyllum inophyllum*, or locally known as Nyamplung, is a plant endemic to Indonesia. Its seed has been documented to contain compounds such as xanthon, coumarins, triterpenoid and flavonoids, which makes *Calophyllum inophyllum* seed extract a potential anti-inflammatory agent to treat inflammatory skin disease. A study conducted by Tsai et al., showed that *C. inophyllum* seed oil was capable of suppressing and stimulate the down expression of nitric oxide production, iNOS, COX-2, and NF- κB in a dose-dependent manner (Tsai et al., 2012) due to the presence of friedelin and triterpene group in C. inophyllum (Shanmugapriya et al., 2016). With everything that has been stated previously, this leads to the primary objective of this research: To investigate the anti-inflammatory effects of *Calophyllum inophyllum* seed ethanol, methanol and hexane extract in TNF-α induced human keratinocyte skin cells (HaCaT).

1.2. Aim and Objective

The aim of this study is to investigate the anti-inflammatory effects of *Calophyllum inophyllum* seed ethanolic extract in TNF-α induced human keratinocyte skin cells (HaCaT). The objectives of this study are as follows: (1) Extraction of *Calophyllum inophyllum* seed using Ethanol, Methanol and n-Hexane as solvents. (2) Determination of *Calophyllum inophyllum*

ethanol, methanol and n-hexane extract concentration which is non-toxic for HaCaT cells. (3) Investigate the anti-inflammatory effects of *Calophyllum inophyllum* ethanol, methanol and n-hexane extract. (4) Elucidate the molecular mechanism of anti-inflammatory effects of *Calophyllum inophyllum* ethanol, methanol and n-hexane extract.

1.3. Scope of Work

The scope of work for this study covers entirely beginning from the preparation of the *Calophyllum inophyllum* Seed Extract preparation, cytotoxicity assay and anti-inflammatory assays. The details of the scope of work for this project are as follows:

1. Preparation of *Calophyllum inophyllum* Seed Extract.
2. *Calophyllum inophyllum* seed extract purification and separation using liquid-liquid fractionation.
3. Cell Culture of human keratinocyte skin (HaCaT) cells.
4. MTT Assay of TNF-α stimulated HaCaT cells treated with *Calophyllum inophyllum* ethanol, methanol and n-hexane extract.
5. RNA Extraction and Quantitative Real Time PCR of pro-inflammatory cytokines.
6. Statistical Analysis.