Chapter 1

Introduction

1.1 Background

Reactive Oxygen Species (ROS) are natural byproducts composed from cellular metabolism (Pizzino et al., 2017). In normal level, ROS play an essential role in the regulation of cellular homeostasis. The intracellular ROS, which are normally in a form of superoxide, level are maintained through the neutralization process by our antioxidants through the expression of antioxidant enzymes such as SOD and GPX. These antioxidant enzymes are able to convert the reactive form of ROS into a more stabilized form (Rolt & Cox, 2020). However, this balance can be disrupted due to various intrinsic factors such as genetics and metabolism; and extrinsic factors such as UV-radiation, alcohol, tobacco smoking, and drugs. This leads to ROS accumulation which overwhelms the natural antioxidant capacity of our body which leads to oxidative stress and causing cell death and eventually aging (Zhang & Duan, 2018). Thus, it is important for us to find a way to increase the natural antioxidant activity in our cell in response to ROS accumulation.

These days, the study of natural-based compounds has become a high demand due to its safer characteristics (Hasanuzzaman et al., 2020). Bryophytes grow and can be seen in a high amount of mosses and liverwort everywhere. Additionally, Indonesia is a country with a high humidity which is suitable for natural plants to grow, including Bryophytes. This plant is known to produce a variety of phytochemicals or secondary metabolites that have been widely used in research, which are phenolic compounds and alkaloids. Flavonoids, a diverse class of phenolic compounds, have been known to protect biological processes from the damaging effects of oxidative stress. Interestingly, bryophytes have been reported to have a high flavonoid content that has been utilized for antioxidant and anti-inflammatory purposes (Ullah et al., 2020). There have been many studies on bryophytes species about its antibacterial activity due to its high amount of secondary metabolites; however, the

antioxidant aspect of this plant is yet to be explored. One of the understudied species of Bryophytes that is indigenous to Indonesia is *Pogonatum neesiii*. Additionally, our preliminary study also shows that our extract, that is *Pogonatum neesiii* actually has no cytotoxic effect towards the HaCaT cell which is safe for other research purposes (unpublished).

1.2 Objective

The objective of this study is to evaluate *P. neesiii* extract potential in inducing the gene responsible for antioxidant properties in the HaCaT cells. First, the bioactive compound of *P. neesii* will be investigated through qualitative and quantitative screening. Then its antioxidant properties will then be tested using DPPH assay while the gene expression ability will be conducted using qRT-PCR towards *GPX1* and *SOD2* which are responsible to neutralize ROS.

The activities that will be carried out in this project includes the extract characterization of *P. neesii* along with HaCaT cells culturing. The extract was then treated to the HaCaT cell and checked for its cytotoxic and cytoprotective activity using MTS assay. DPPH assay will be performed to check the antioxidant activity of *P.neesii.* Primers will be designed along with processing the extraction of RNA. The expression of genes responsible for antioxidant activity (*GPX1* and *SOD2*) will be done using qRT-PCR, and lastly statistical analysis.

1.3 Hypothesis

This project hypothesizes that *P. neesii* extract contains bioactive compounds that can induce the expression of *GPX1* and *SOD2* in which it exhibit antioxidant properties towards HaCaT cells