Chapter 1

Introduction

1.1 Research Background

Reactive oxygen species (ROS) is a form of free radicals that is highly reactive and derived from both internal and external sources (Nakai & Tsuruta, 2021). Sources of ROS from within the body come from various non-enzymatic processes, such as aerobic metabolism, while external sources include air pollution, cigarette smoking, UV exposure, and industrial chemicals (Pham-Huy et al., 2008; Lobo et al., 2010). As a byproduct of aerobic metabolism, ROS plays essential roles in the body, including cell signaling, cell survival, apoptosis modulation, and inflammation-related factor production. Under basal conditions, antioxidants and pro-oxidant molecules must coexist in balance to promote the effectiveness of antioxidant defense activity. Conversely, an imbalance between antioxidants and pro-oxidant molecules leads to oxidative stress that can damage the cell. Oxidative stress has the ability to cause direct damage to relevant cell molecules, including DNA, proteins, and lipids, which then interfere with the transcriptional regulation of genes (Letsiou et al., 2017; Lobo et al., 2010).

A number of research studies have demonstrated that antioxidants are capable of preventing tissue damage caused by free radicals through direct or indirect mechanisms. Antioxidants can reduce oxidative damage indirectly by increasing the activity or expression of intracellular antioxidant enzymes or directly by interacting with free radicals (Lobo et al., 2010; Lü et al., 2010). The most prominent mechanism of antioxidant action is enhancing the expression of antioxidant enzymes, wherein the primary antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GPX), are expressed to stabilize or deactivate free radicals before they attack cellular components. SOD catalyzes the dismutation of superoxide, a highly reactive oxygen species, into hydrogen peroxide (H_2O_2) and oxygen (O_2). GPX then catalyzes the reduction of H_2O_2 to water through

the reduction of glutathione (GSH) to oxidized glutathione (GSSG), therefore protecting the cells against oxidative damage (Patlolla et al., 2009).

Recently, natural products have attracted much interest in discovering their antioxidant properties. Among the sources, plants are a highly studied natural resource for discovering new anti-aging ingredients. One of the plants with potential sources of natural antioxidants is a plant from the genus *Litsea*. Parts of the *Litsea* plant have been used to manage numerous diseases due to the various compounds contained in it. However, more than three-quarters of plant species have not been well studied for their pharmacological effects or phytochemical studies, which makes them promising candidates for further research (Wang et al., 2016). In addition, several plant extracts from the genus *Litsea* have been shown to exhibit pharmacological properties, such as antimicrobial and antioxidant, which reveals a potential application in cosmetics (Wong et al., 2014). For example, a study by Wang et al. (2016) reported that *Litsea coreana* exhibits vigorous antioxidant activity due to the presence of flavonoids. Wong et al. (2014) also reported that the methanol extract of *Litsea resinosa* has a high antioxidant activity at DPPH, with an IC_{50} of 11.22 mg/mL. Moreover, Kamle et al. (2019) recently presented that an extract obtained from *Litsea cubeba* had anti-cancer, anti-inflammatory, antimicrobial, antioxidant, antidiabetic, and anti-HIV activity.

Since these findings point towards the antioxidant activity of the genus *Listea*, *Litsea oppositifolia* was chosen in this study to be evaluated for potential anti-aging application. *L. oppositifolia* has never been studied for its pharmacological role at the cellular level under H_2O_2-induced conditions, which is very interesting to be studied. Therefore, this study was conducted in order to investigate the effect of *Litsea oppositifolia* stem extract with antioxidative properties using keratinocytes HaCaT cell line.

In this study, the stem extract of *L. oppositifolia* was selected because it possessed the highest antioxidant activity against DPPH free radicals, as revealed in a previous study by Zakhrifah

(unpublished, 2019) in which the IC_{50} value was 8.310 ± 0.04 µg/mL. The cytotoxic activity and cytoprotective ability of *L. oppositifolia* stem extract were evaluated *in vitro* using the HaCaT cell line, which is representative of the human epidermis, employing a cell viability assay. Furthermore, qRT-PCR was also performed to evaluate the expression of SOD-2 and GPX-1, whether the plant extract could upregulate or downregulate their expression.

1.2 Objectives

The key objective of the present research is to analyze the capability of *L. oppositifolia* stem extract in providing an antioxidant effect against oxidative models on HaCaT cells. The specific aims of this study are elaborated below:

a. Investigate the radical scavenging ability of *L. oppositifolia* stem extract.
b. Evaluate the cytotoxic effect of *L. oppositifolia* stem extract towards HaCaT cell line.
c. Investigate cytoprotective capability of *L. oppositifolia* stem extract on HaCaT cells treated with H_2O_2 as oxidative stress model.
d. Investigate the role of *L. oppositifolia* stem extract in the expression of SOD-2 and GPX-1 genes under the effect of ROS-induced damage.

1.3 Hypothesis

- *L. oppositifolia* stem extract contains phytochemical constituents such as flavonoid and phenols.
- *L. oppositifolia* stem extract is able to scavenge DPPH free radicals.
- *L. oppositifolia* stem extract possesses low toxicity on HaCaT cells
- *L. oppositifolia* stem extract possesses cytoprotective capability against oxidative stress model induction.
- *L. oppositifolia* stem extract enhances the expression of SOD-2 and GPX-1 as a response to ROS.