Chapter 1

Introduction

1.1 Background

Skin aging is a process in which the skin declines in quality that can be affected by intrinsic and extrinsic factors, typically denoted by the appearance of folds and wrinkles. The intrinsic factors of aging are attributed to physiological changes, such as genetic mutation, metabolism, and hormones. Meanwhile, extrinsic aging is promoted by exposure to environmental factors, namely ultraviolet (UV) radiation, pollutants, and toxins (Chaudary, Khan, & Gupta, 2020; Zhang & Duan, 2018). Due to the inevitable nature of human skin aging, many people spend considerable expenses to purchase skincare products that can prevent the aging process. According to Fortune Business Insight (2018), it was discovered that the global anti-aging market size reached a value of USD 38.62 billion in 2018. This number was expected to escalate, and by 2026, it was expected that the value would reach 60.26 billion USD. Thus, the high demand for anti-aging cosmetics renders the opportunity to compete in the market by researching new anti-aging treatments (Zhang & Duan, 2018).

The aging process occurs due to several mechanisms, and one of the factors is due to the generation of ROS in the skin, such as the superoxide anion (O^{2-}), hydroxyl radical (OH), and hydrogen peroxide (H_2O_2) (Xiao et al., 2020). ROS is endogenously formed by the body during inflammation to fight against invading microorganisms and is continuously produced at low levels in the skin (Xu et al., 2017). To prevent excessive oxidative processes, the body also undergoes antioxidant processes. However, excessive amounts of ROS may overwhelm the body's antioxidant mechanism, which can lead to oxidative stress that is associated with skin aging by degrading the extracellular matrix (ECM) that leads to the formation of wrinkles, which is a prominent characteristic of premature aging (Xu et al., 2017; Tsuchida & Kobayashi, 2020; Habib et al., 2013). Furthermore, excess ROS can be induced by external factors such as UV, pollutants, and chemicals. Therefore, to counteract this problem, the development of products that could protect the skin from oxidative stress is necessary (Oresajo et al., 2012).

Due to the natural cosmetics trend, which became popular in 2015, resulting in 10-11% annual market growth, the use of botanical extracts in anti-aging cosmetics has been highly researched to fulfill the market demand (Ferreira et al., 2021). As a result, many plants have been studied for their anti-aging potential by acting as an antioxidant agent, one of which is the *Litsea spp*. *Litsea* is a genus of evergreen trees belonging to the Lauraceae family, which encompasses many species found in tropical and subtropical regions of Asia and North America. From past research, several species from the *Litsea* genus have been found to exert antioxidant effect (Ambarwati et al., 2021; Wong et al., 2014). However, some *Litsea* species have not been extensively studied for their antioxidant effects, such as *Litsea oppositifolia*, which is native to Borneo. Previously, the antioxidant potential of *Litsea oppositifolia* stem extract has been assessed via DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric-reducing antioxidant power) assays which showed that the extract could be a good candidate for a natural antioxidant source (Zakhrifah, 2019). However, there is still no study on the effects of the extract on skin cells yet, and therefore, evaluation of the effects of *Litsea oppositifolia* extract on cells is still needed before incorporation of the compound into products.

Aside from physical appearance observation, the signs of aging can also be marked by fluctuations in gene expression. ROS accumulation induces the activation of inflammatory pathways by activating transcription factors such as the nuclear factor-kB (NF-kB). The stimuli cause the translocation of NF-kB into the nucleus and bind with the cyclooxygenase-2 (COX-2) promoter, an enzyme responsible for prostaglandins' production, resulting in its upregulation and promoting inflammation (Park et al., 2019). Following the inflammation process, subsequent ECM degradation occurs, mediated by the MMPs family. The matrix metalloproteinase-1 (MMP-1) gene is involved in the breakdown of extracellular matrices during normal physiological processes (e.g. tissue remodeling, reproduction, aging, and embryonic development). A research conducted by Kim et al. (2012) found that the generation of ROS could induce the synthesis of MMP-1, leading to the stimulation of the aging phenomenon. The MMP-1 gene promotes aging by degradation of type I collagen, the most abundant structural protein in the skin's connective tissues (Wang, 2014). Therefore, analyzing the expression of COX-2 and MMP-1 can determine the antioxidant effects by observing the changes in gene expressions.

In this study, DPPH assay was performed to determine the radical scavenging capability of the extract *in vitro*. Furthermore, the antioxidant effect of *Litsea oppositifolia* stem extract was evaluated at cellular level through cytoprotective assay, where the extract was expected to protect the cells when challenged against H_2O_2 through radical scavenging activity. The gene expression analysis of COX-2 and MMP-1 genes, which are responsible for skin aging, was also conducted to determine whether the extract could mediate skin aging at the molecular level, where the downregulation of both genes indicated that the extract could protect the cells by controlling the expression of genes related to skin aging,

1.2 Objective

This study aimed to analyze the protective capability of the extract in HaCaT cells by conducting *in vitro* cytoprotective activity through the measurement of cell viability after treatment with H_2O_2 using MTS assay, and gene expression analysis of COX-2 and MMP-1 through qPCR. Additionally, a phytochemical screening, total flavonoid, total phenolic content, total alkaloid, and DPPH assay were performed to characterize the extract and relate them to the protective capability.

1.3 Hypothesis

The hypotheses for this research were:

- The *Litsea oppositifolia* stem extract contains phytochemicals such as phenols and flavonoids
- The *Litsea oppositifolia* stem extract has protective effect toward HaCaT cells when challenged with H_2O_2
- The *Litsea oppositifolia* stem extract can exert antioxidant activity through radical scavenging and by directly downregulating gene expressions at molecular level, which results in the downregulation of COX-2 and MMP-1 expressions