Chapter 1
Introduction

1.1 Background

Skin aging is a natural biological process characterized by a gradual decline in the structural and functional properties of the skin. This process is influenced by both genetic factors and environmental exposures (Naidoo et al., 2017). Among environmental factors, ultraviolet (UV) radiation, particularly ultraviolet B (UVB), is considered the most harmful and represents the primary cause of photoaging, which is estimated to account for nearly 80% of visible facial aging (Gromkowska-Kępka et al., 2021; Xie et al., 2024). A key feature of skin aging is the imbalance of the synthesis and breakdown of extracellular matrix (ECM) components. This degradation is largely driven by matrix metalloproteinases (MMPs), and UV exposure has been reported to increase collagen breakdown up to threefold through enhanced MMP activity (Feng et al., 2024). Within the MMP family, MMP-1 and MMP-9 are particularly important in skin aging because of their specific roles in degrading collagen and elastin fibers (Im et al., 2015; Tang et al., 2025).

MMP activation occurs via multiple mechanisms, with tumor necrosis factor-α (TNF-α) signaling serving as a major regulator. TNF-α is released rapidly following ultraviolet radiation (UVR) exposure (Pocino et al., 2024). Consequently, increased TNF-α levels can promote elevated expression of various MMP enzymes. MMP-1, also known as collagenases, is regarded as a key enzyme in skin aging among all MMP types as it selectively breaks down collagen types I and III in the skin (Tang et al., 2025). When skin ages, triple-helical collagen molecules are broken down by MMP-1 into denatured fibrils, which are then broken down by gelatinases such as MMP-9 (Mixon et al., 2022). Therefore, collagenases like MMP-1 and gelatinases like MMP-9 are the main causes of skin aging based on the process of collagen degradation in skin aging. Together, these enzyme-mediated processes contribute to ECM degradation and play a central role in UVR-induced skin aging.

Although skin aging cannot be completely prevented, its progression can be slowed through cosmetic interventions, with natural extracts with high phenolic molecules gaining popularity (Nisa et al., 2024;

Xie et al., 2024). They can modulate signaling pathways involved in skin protection against UV damage, lower oxidative stress, and also act as MMPs inhibitors (MMPIs) (Jian et al., 2022; Petruk et al., 2018). Among medicinal plants, *Physalis angulata* (*P. angulata*), or known as Ciplukan, and *Vitex trifolia* (*V. trifolia*), known as Legundi, are recognized for their high levels of phenolic metabolites, which may contribute to anti-aging properties (Sistyananda et al., 2026). However, to date, no studies have comprehensively investigated the anti-aging mechanisms of these plants, particularly their potential to inhibit MMPs activity and expression. It is known that *P. angulata* has a high concentration of chlorogenic acid (CGA), caffeic acid (CA), p-coumaric acid, rutin, and quercetin (Nguyen et al., 2021). Likewise, *V. trifolia* contains high total phenolic and flavonoid content, which are casticin, chrysosplenol D (CHD), luteolin, vitexin, and apigenin, which may explain its antioxidant activity (Fawwaz et al., 2024; Nisa et al., 2023). While *P. angulata* and *V. trifolia* have long been utilized for active ingredients in traditional medicine, their potential applications in skincare and anti-aging research also remain limited (Khat-Udomkiri & Petsringoen, 2025). However, it has been demonstrated that a number of compounds present in *P. angulata* and *V. trifolia* inhibit the expression of MMP-1 and MMP-9. Collectively, these MMP expression inhibitions result in *P. angulata* and *V. trifolia* having a strong potential for photoaging mitigation.

In silico approaches are essential in this study as a preliminary evaluation of natural compounds from *P. angulata* and *V. trifolia* against key photoaging-related targets, namely MMP-1, MMP-9, and TNF-α. The results of the *in silico* analysis will be validated through *in vitro* experiments, including qRT-PCR to assess gene expression (Dana et al., 2020). Moreover, the limited *in silico* studies examining these plants in relation to MMP-1, MMP-9, and TNF-α highlight a research gap and support the need for this investigation to confirm their anti-aging potential. Therefore, this study aims to evaluate the anti-aging effects of *P. angulata* and *V. trifolia* by performing an *in silico* analysis of the phenolic and flavonoid compounds from both plants against MMP-1, MMP-9, and TNF-α, as well as measuring the gene expression levels in UV-induced human keratinocyte HaCaT cells.

1.2 Objective

The aim of this research is to investigate the anti-aging activity of *P. angulata and V. trifolia* by performing *in silico* analysis of CGA, CA, p-coumaric acid, rutin, and quercetin in *P. angulata* and casticin, CHD, luteolin, vitexin, and apigenin in *V. trifolia* against MMP-1, MMP-9, and TNF-α. Moreover, the gene expression levels of MMP-9 and MMP-1 in UV-induced human keratinocyte HaCaT cells through qRT-PCR would also be measured.

1.3 Hypothesis

For the *in silico* analysis, the hypotheses are:

H0: Natural compounds from *P. angulata* and *V. trifolia* do not exhibit a strong binding affinity against MMP-1, MMP-9, and TNF-α.

H1: Natural compounds from *P. angulata* and *V. trifolia* exhibit a strong binding affinity against MMP-1, MMP-9, and TNF-α.

For the gene expression analysis using *P. angulata* treatment, the hypotheses are:

H0: *P. angulata* extract has no significant effect on the MMP-1 and MMP-9 gene expression inhibition in UVB-induced HaCaT cells.

H1: *P. angulata* extract has significant effect on the MMP-1 and MMP-9gene expression inhibition in UVB-induced HaCaT cells.

For the gene expression analysis using *V. trifolia* treatment, the hypotheses are:

H0: *V. trifolia* extract has no significant effect on the MMP-1 and MMP-9 gene expression inhibition in UVB-induced HaCaT cells.

H1: *V. trifolia* extract has significant effect on the MMP-1 and MMP-9gene expression inhibition in UVB-induced HaCaT cells.