Chapter 1

Introduction

1.1. Background

Inflammation is the body's initial response towards injury, harmful irritants, and pathogens that enter the body, with the main importance of wound healing and recovery. The stimuli can be in the form of damage-associated molecular patterns (DAMPs) produced by internal damaged cells or pathogen-associated molecular patterns (PAMPs) from infectious agents. The acute inflammation is the most common type of inflammation, characterized by erythema (redness), swelling, heat, pain, and loss of function (Furman et al., 2019; Institute for Quality and Efficiency in Health Care, 2021). Acute inflammation is an immediate innate response against the stimuli that lasts for several days, primarily mediated by pattern recognition receptors (PRRs), located on macrophages, dendritic cells, and other immune cells (Sun et al., 2012; Chelangarimiyandoab et al., 2025).

Lipopolysaccharide (LPS) is a well-characterized PAMP used to induce inflammation in experimental *in vitro* models through the TLR4 receptor and NF-κB pathway, increasing the expression and production of cytokines, especially TNF-α and IL-6 (Bertani & Ruiz, 2018). Cytokines are small intercellular signaling proteins (<40 kDA) secreted to regulate and influence the immune system and inflammatory response (Liu et al., 2021). Prolonged acute inflammation leads to the development of chronic inflammation as the immune system continues to produce white blood cells and cytokines, disrupting the body's recovery pathways. This chronic inflammation can last up to several months and years while increasing the risk of developing autoimmune conditions, cancer, cardiovascular disease, metabolic disorders, and skin disease (Chavda et al., 2025).

Perilla *(Perilla frutescens)* leaves are one of the first acknowledged medicinal and edible plants. Despite the different varieties of perilla leaves, the high concentration of bioactive compounds enables therapeutic benefits, such as antioxidant, antibacterial, antimutagenicity, anti-inflammatory,

anti-tumor, and antiallergic properties (Kim et al., 2024). The key players in the anti-inflammatory activity are perillaldehyde, luteolin, rosmarinic acid, and apigenin (Kagawa et al., 2019; Tantipaiboonwong et al., 2023; Zhou et al., 2024). Studies have shown anti-inflammatory effects from each key component in perilla leaves. Perillaldehyde downregulates the expression p-IκB and inhibits the cGAS/STING-mediated IRF3/NF-κB pathway (Wei et al., 2024). Luteolin inhibits the TLR4 receptor and NF-κB signaling pathway (Sun et al., 2025). Rosmarinic acid inhibits the NF-κB pathway and activates PPAR-γ (Luo et al., 2020). Apigenin acts as an IRAK4 inhibitor and prevents IκB degradation (Gongpan et al., 2025; Salehi et al., 2019). Ultimately, these compounds downregulates the inflammatory response, resulting in less production of pro-inflammatory cytokines and nitric oxide (NO).

Although the anti-inflammatory properties of *Perilla frutescens* leaf have been studied in LPS-induced RAW264.7 cells, research gaps exist prior to this study. Hence, this study was conducted to bridge the gaps. First, most studies utilized fresh and research-grade perilla leaves (Tantipaiboonwong et al., 2023). Consequently, the anti-inflammatory activity of commercially available dried perilla leaves in the Indonesian market has not been extensively studied. Hence, geographic variation may influence the phytochemical constituents of perilla leaves (Kang et al., 2025). Second, this study implemented a continuous study design using the same LPS-stimulated cells and supernatant for WST-8 cell viability and ELISA cytokine quantification, to assess the interaction between LPS and extract in regard to cell viability and evaluate the extract's anti-inflammatory activity during an active inflammatory state, as LPS cellular metabolism and may shift cytotoxicity thresholds. Third, the dried perilla leaf extraction methods vary across literature, especially regarding the solvent type. This study performed maceration with 80% methanol as journals utilizing plant extract showed high extraction efficiency for anti-inflammatory compounds (Ashenafi et al., 2023; Lee et al., 2024).

1.2. Objective

This study aims to evaluate the anti-inflammatory effects of dried perilla leaves (*Perilla frutescens*) extract by characterizing the extract, investigating its cytotoxic effects, and quantifying the expression and production of TNF-α and IL-6 cytokines in LPS-induced RAW264.7 cells.

1.3. Research Question

1. Does FTIR analysis on *Perilla frutescens* leaves extract confirms the presence of functional groups associated with anti-inflammatory compounds as reported in literature?
2. Do dried *Perilla frutescens* leaves extract administered within the safe dosage range be able to reduce the expression and production of TNF-α and IL-6 by LPS-induced RAW264.7 cells?

1.4. Hypothesis

$H0_1$: FTIR analysis of *Perilla frutescens* leaf extract does not confirm the presence of functional groups associated with anti-inflammatory compounds as reported in literature.

$H1_1$: FTIR analysis of *Perilla frutescens* leaf extract does confirm the presence of functional groups associated with anti-inflammatory compounds as reported in literature.

$H0_2$: There is no significant difference ($p > 0.05$) in TNF-α and IL-6 expression and production by LPS-induced RAW264.7 cells treated with and without dried *Perilla frutescens* leaf extract.

$H1_2$: There is significant difference ($p < 0.05$) in TNF-α and IL-6 expression and production by LPS-induced RAW264.7 cells treated with and without dried *Perilla frutescens* leaf extract.