Chapter 1

Introduction

1.1 Background

Inflammation is the body's self-defense against pathogens, tissue damage, and cellular stress. It is a basic biological response that is necessary and important for the body (Oronsky et al., 2022). The inflammation process consists of several processes that work together to eliminate the infection, dead cells and tissues, and create an environment that is optimal for repair and growth (Antonelli & Kushner, 2017).

There are two types of inflammation, acute and chronic. According to Pahwa et al. (2023), acute inflammation is the body's quick, general response, and it usually lasts for a few days to a few weeks. It involves immune cells such as macrophages and mast cells, which release vasoactive amines, eicosanoids, and pro-inflammatory cytokines. This signals the neutrophils and monocytes from the blood to the site of injury, facilitating phagocytosis. Tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-1α (IL-1α), interleukin-1β (IL-1β), and interleukin-18 (IL-18) are early pro-inflammatory cytokines that are secreted by activated macrophages and induce an inflammatory signal. Certain cytokines, such as interferon-gamma (IFN-γ) and TNF-α, signal the inducible Nitric Oxide Synthase (iNOS) to be upregulated in the macrophage, which leads to the increased production of Nitric Oxide (NO) (Samir et al., 2017).

Meanwhile, chronic inflammation is a prolonged, dysregulated immune response that lasts for months to years. This state involves mononuclear cells, such as macrophages and lymphocytes, as well as the continuous release of pro-inflammatory mediators, including pro-inflammatory cytokines, chemokines, and reactive oxygen species (Germolec et al., 2018). While acute inflammation protects the body and prevents damage from happening, chronic inflammation, on the other hand, causes tissue damage and poor cell repair, which could lead to inflammatory diseases (Arida et al., 2018). In these cases, the immune system becomes part of the illness. In this state, TNF-α and IFN-γ become

the primary molecules that maintain the inflammatory signals, stimulating cells to produce enzymes such as iNOS (Taveepanich et al., 2024). This enzyme continuously releases NO, which, in large amounts, damages the tissues.

Studies show that diets abundant in polyphenols and antioxidants correlate with reduced symptoms of inflammation and lower rates of inflammatory diseases. This is because these compounds can inhibit transcription factors such as Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and Nuclear factor erythroid 2-related factor 2 (Nrf2), which control the expression of inflammatory genes (Zhang & Tsao, 2016). *Caesalpinia sappan*, also known as Sappan wood, is a plant that has been used for traditional medicine in Southeast Asia. Ancestors have used the heartwood as a natural dye and as a medicine (Vij et al., 2023). It has linked benefits to the heart, liver, spleen meridians, and circulation, as well as reducing pain and swelling (Wirawati et al., 2025). One of the main bioactive compounds in sappan wood is brazilin, a homoisoflavanoid that gives the plant its red pigment. Previous studies have proven the various biological functions of brazilin, such as acting as a strong antioxidant, antimicrobial, hepatoprotective, and specifically, anti-inflammatory properties (Taveepanich et al., 2024).

However, there is still a gap in the current research. Most studies have focused on isolated brazilin or other bioactive compounds present in *Caesalpinia sappan* (Taveepanich et al., 2024; Wu et al., 2011), which is valuable for identifying specific bioactive compounds, yet it overlooks the other compounds present in the crude extract. Sappan wood contains a wide range of other phenolic compounds, which may contribute synergistically to the overall anti-inflammatory effects (Vij et al., 2023). Additionally, a comprehensive evaluation integrating cell viability, inflammatory mediators (NO, TNF-α, IL-6), and gene expression in a single study is still limited. Therefore, further investigation of crude *Caesalpinia sappan* extract is needed to better understand its anti-inflammatory potential.

1.2 Objective

This study aims to characterize *Caesalpinia sappan* extract using Fourier Transform Infrared (FTIR) spectroscopy and investigate its anti-inflammatory potential using an *in-vitro* model of LPS-induced inflammation in RAW264.7 cells. The study will analyze the extract's effect on cell viability, the expression of inflammatory genes (*Tnf-α*, *Il-6*, and *Nos2*), the secretion of pro-inflammatory cytokines (TNF-α and IL-6), and nitric oxide (NO) production.

1.3 Research Questions

1. Does the FTIR analysis of *Caesalpinia sappan* extract confirm the presence of functional groups consistent with its known bioactive compounds, as reported in the literature?
2. Does *Caesalpinia sappan* extract maintain cell viability and exhibit anti-inflammatory effects by reducing the expression of inflammatory genes (*Tnf-α*, *Il-6*, and *Nos2*), decreasing the secretion of pro-inflammatory cytokines (TNF-α and IL-6), and inhibiting nitric oxide (NO) production in LPS-induced RAW264.7 cells?

1.4 Hypothesis

$H0_1$: FTIR analysis of *Caesalpinia sappan* extract will not confirm the presence of functional groups consistent with its known bioactive compounds as reported in the literature.

$H1_1$: FTIR analysis of *Caesalpinia sappan* extract will confirm the presence of characteristic functional groups that are consistent with the major bioactive compounds reported in the literature.

$H0_2$: Treatment with *Caesalpinia sappan* extract will have no significant effect on cell viability, inflammatory gene expression (*Tnf-α*, *Il-6*, and *Nos2*), pro-inflammatory cytokine secretion (TNF-α and IL-6), or Nitric Oxide (NO) production in LPS-induced RAW264.7 cells.

$H1_2$: Treatment with *Caesalpinia sappan* extract will maintain cell viability and significantly reduce inflammatory gene expression (*Tnf-α*, *Il-6*, and *Nos2*), pro-inflammatory cytokine secretion (TNF-α and IL-6), and Nitric Oxide (NO) production in LPS-induced RAW264.7 cells.