

I. INTRODUCTION

1.1 Project Background

Inflammation is defined as the natural response of the immune system to several stimuli via some factors including physical, chemical noxious stimuli or microbiological toxins. The fundamental purpose of this inflammatory response is to eliminate irritants, neutralize invading pathogens, and set the stage for tissue repair and healing. While inflammation plays an indispensable role in the body's defense mechanisms, prolonged or intense inflammation can trigger a spectrum of health complications. These encompass autoimmune disorders, cardiovascular diseases, neurological impairments, and even certain forms of cancer (Dzoyem et al., 2017).

Candlenut (*Aleurites moluccanus*) is widely known as a culinary spice and herb especially it can be easily found in Indonesia and Southeast Asia regions. It has been traditionally used in various cuisines, particularly in Indonesia, made into a paste to make sauces, dressings, or as a base for certain dishes (de Britto Rosa et al., 2022). Renowned for its culinary applications, cold-pressed candlenut oil boasts a notable oil content, predominantly composed of polyunsaturated fatty acids (PUFAs). In particular, the presence of omega-3 fatty acids contributes to the regulation of lipid metabolism within the body. This regulation is achieved through the elevation of high-density lipoprotein (HDL) cholesterol while concurrently reducing low-density lipoprotein (LDL) cholesterol. This modulation is pivotal in curtailing atherosclerotic progression. Moreover, a study from Wang et al., (2018) claimed that Candlenut has the largest antioxidant and polyphenol compared with other legumes.

According to (Hussain et al., 2016), PUFA and polyphenols have several anti-inflammatory and antibiotic properties and may in conjunction with the activation of the transcription factor

Nrf2. Nrf2 take part a crucial role in cellular defense towards oxidative stress and inflammation. Therefore, Candlenut's remarkable attributes of PUFA and phenolic compounds extend to its significant anti-inflammatory properties (Yusri et al., 2020). Subsequently, the focus of this research lies in exploring the anti-inflammatory attributes of Candlenut aqueous extract through its application *in vitro*.

The methodology used is cell culture employing the RAW264.7 macrophage and applying lipopolysaccharide (LPS) for inflammatory induction. LPS constitutes a segment of the outer membrane of Gram-negative bacteria. These bacteria are frequently employed *in vitro* to create models for studying macrophage inflammation and evaluating the potential anti-inflammatory properties of naturally occurring compounds. LPS stimulation prompts the production of pro-inflammatory cytokines, including tumor necrosis factor (TNF- α) and IL-6 (Dong et al., 2017). Therefore, the lipopolysaccharide stimulated RAW 264.7 cells in addition of the treatments involving diverse concentrations of Candlenut ethanol extract (CEE) is analyze of its effect in the anti-inflammatory level cytokines IL-6 and TNF- α by ELISA for its protein expression (Ishida et al., 2019).

1.2 Objectives

The objectives of this study includes:

- To examine the anti-inflammatory properties of Candlenut ethanol extract *in vitro*.
- To examine the various concentrations of Candlenut ethanol extract to the anti-inflammatory effect.

1.3 Scope of the Project

- Conduct sample preparation, extraction using ethanol, and plating to 96-well plate.

- Perform cell culture using RAW264.7 and carry out several treatments such as cell treatment with LPS and incorporation of CEE.
- Assess the cytotoxicity of various concentrations of CEE (200 µg/mL, 100 µg/mL, 50µg/mL, 25µg/mL, and 12.5µg/mL) to RAW264.7 cells using cell viability assay (WST-8 assay).
- Examine the anti-inflammatory characteristic of CEE, specifically the production of anti-inflammatory cytokines of IL-6 and TNF-α that is quantified using Enzyme-linked immunosorbent assay (ELISA).

1.4 Problem Formulation

Refer to the specified objectives and background, the research questions that will be analyzed in this report is:

- Is there any correlation between Candlenut and anti-inflammatory effect?
- What is the effect of using different concentrations of CEE (200 µg/mL, 100 µg/mL, 50µg/mL, 25µg/mL, and 12.5µg/mL) on it's anti-inflammatory potential?

1.5 Hypothesis

H0 : CEE shows anti-inflammatory potential.

H1 : CEE does not show anti-inflammatory potential.

H0 : The anti-inflammatory potential of CEE is in a dose-dependent manner.

H1 : The anti-inflammatory potential of CEE is not in a dose-dependent manner.