CHAPTER 1. INTRODUCTION

1.1. Project Background

In the present time, herbal medicine derived from natural products is extensively used in both developing and developed countries (Sholikhah, 2016). Numerous traditional medicines from various cultures in the world used medicinal herbs as the backbone of their health care system (Wachtel-Galor & Benzie, 2021). In Indonesia, the use of traditional medicine for various diseases has been passed down for generations since centuries ago as a part of national culture. As a megadiverse country, Indonesia is endowed with abundant natural resources in terms of plants and spices that are broadly known by Indonesian people as a cure for various diseases (Sholikhah, 2016). With the advancement in science and technology as well as the growing market for herbal medicine, it is crucial to scientifically establish the efficacy of traditional medicine existing in the market to further explore, research, develop and optimize for their utilization as herbal medicine.

Herbal medicine is believed to be able to act as an immunomodulator and treat many immune-related diseases such as systemic or exocrine autoimmune and inflammatory manifestations, which are mainly lacking on a scientific basis. In the last few years, the immunomodulatory activity of plants and plant extracts are being examined widely globally. Various plants and herbs used in traditional medicine utilize their antimicrobial properties not only by directly targeting the disease-causing pathogens but also by enhancing the natural and adaptive immunity of the host (Shukla, Bajpai & Kim, 2012). Several plants have been extensively studied regarding their immunomodulatory effect compared to others, namely several *Phyllanthus species, particularly Phyllanthus amarus, Phyllanthus emblica, Phyllanthus niruri, and Phyllanthus urinaria* (Jantan, Haque, Ilangkovan & Arshad, 2019). *Phyllanthus* species have been

established to have significant immunomodulatory activity toward both innate and adaptive immunity (Jantan, Haque, Ilangkovan & Arshad, 2019). It is well established in many pre-clinical studies that *Phyllanthus* species, in particular, *Phyllanthus niruri L.* is able to modulate the immune system by increasing the proliferation rate and activation of T cells and B cell, secretion of specific cytokines, and enhancement in the phagocytic activity, increasing the cytotoxicity of natural killer cells, as well as activation of the complement system (Tjandrawinata & Nofiarny, 2005).

Immunomodulators are becoming well-known in the natural health market as people started to recognize the significance of a healthy immune system in the maintenance of health as prophylaxis and curative agent (Srikumar, Jeya Parthasarathy & Sheela Devi, 2005). An immunomodulator is an agent that has the ability to modify immune functions in a beneficial way by enhancing (immunostimulators) or decreasing (immunosuppressive) the immune response (Bascones-Martinez et al., 2014). The most frequently used plant-derived immunomodulators is as an immunostimulant to strengthen the immune response against various infectious diseases, tumors, as well as primary or secondary immunodeficiency, which reflects in the claims of commercially available herbal products in the market (Asherson et al., 2009). In the past few years, there has been an upsurge in the clinical use of a certain herbal medicine as an immunomodulator, such as herbal medicine made from *Echinacea*, *Ginkgo biloba*, *Panax*, and *Camellia* (Lewicka et al., 2019; Ortuño-Sahagún, Rawat & Zänker, 2019). As a medicine, the use of herbal products must fulfill the requirements of safety and effectiveness. Therefore, it is crucial to establish the pharmacological effects of herbal medicine through scientific proof. The immunomodulatory activity can be assessed in vitro and in vivo by researching the cells and proteins responsible for the immune system mechanism.

Tamba toya sajiwani is a mixture of traditional Balinese herbal medicine made of 21 different herbal plants that are formulated according to the Ratuning Ushada book and are believed to have many beneficial health effects. In this project, the immunomodulatory activity of herbal drink (Tamba toya sajiwani) on innate immune systems will be assessed for the first time *in vivo* through carbon clearance assay and differential leukocyte count in mice model in order to scientifically prove the

immunomodulatory activity of the herbal drink and establishing the herbal drink as alternative supplementation to conventional treatment as an immunomodulator.

1.2. Research Objectives

Objective of this project is listed below:

● To evaluate the immunomodulatory activity of a traditional herbal drink (Tamba toya sajiwani) on the innate immune system *in vivo*.

● To evaluate the effect of herbal drink on the reticuloendothelial system through carbon clearance assay.

● To determine the leukocyte count of mice after treatment with herbal drink.

1.3. Research Hypothesis

Herbal drink (Tamba toya sajiwani) will have a favorable immunomodulatory effect, in particular an immunostimulant effect in mice.

1.4. Research Scope

This study will focus on the evaluation of the effect of herbal drink (Tamba Toya Sajiwani) administration on mice's immune system. Specifically, the scope of work that would be employed in this study include:

● Carbon clearance assay - evaluate the effect of drugs on the reticuloendothelial system.

   ○ Measure the rate of carbon clearance - through a colorimetric method.

   ○ Measure the phagocytic index - direct measure of macrophage function.

● Leukocyte count assay - analyze the total leukocyte and differential leukocyte to assess the amount of each type of white blood cell.