

CHAPTER I

INTRODUCTION

1.1. Background of Study

The immune system's function is to provide defense of the human body for both infectious diseases and malignancies. Immunity is known as the innate immunity if the response is elicited as a natural mechanism without prior antigen-presenting mechanism, while adaptive immunity is as a result of antigen-presenting mechanism and it stores the memory cells. Thus the secondary recognition creates stronger response than the innate response.¹

Phagocytosis is one of the important mechanisms of innate immunity. This mechanism is an essential first phase of body defense, as a bridge between innate and adaptive immunity, that also takes part as an effector mechanism of adaptive immunity. The main phagocytes or cells that phagocytize are the polymorphonuclear leukocytes such as neutrophils, and mononuclear cells such as macrophages (the residential monocytes that have undergone a differentiation). Macrophages are known as the professional phagocytes, attributed with various innate and unique receptors that recognize microbes. Those cells perform antigens recognition earlier, ingest the antigens and act as the Antigen-Presenting cells (APCs) to the adaptive immune cells.²

Mechanism of phagocytosis is complex and it involves phagocyte cellular mechanism. During the recognition, macrophages identify their antigens via the

receptors such as Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-like receptors (RLRs), C-type lectin-like receptors, N-formyl-Met-Leu-Phe receptors, Mannose receptors, and Scavenger receptors.^{3, 4} Once the receptor recognizes the component of bacterial cell wall, the genes which controlling the cellular response are expressed. The cell membrane is reconstructed to be a cup-shaped projection that surrounds the microbe, and with the coordination of cytoskeletons the engulfment process begins.⁵

The intracellular killing mechanisms of pathogen are by the oxygen-dependent and non oxygen-dependent pathways. The oxygen dependent is mediated by the Reactive Oxygen Species (ROS), while the non-oxygen dependent via the Nitric oxides (NOs). In adaptive immunity, macrophages those are activated by the T Helper lymphocytes subset 1 (T_H1 cells) will increase their secretions of ROS and NOs, thus enhance the effector mechanism (as effector phagocytes).⁶ The macrophages activation is an essential key for the immunity, especially for the phagocytized microbes that possibly undergo evasion during phagolysosome fusion. *Mycobacterium tuberculosis*, *Listeria monocytogenes*, and *Salmonella typhi* are the examples of microbes with ability to evade the immune response. This is underlying the importance of macrophages activation during immune response to kill the phagocytized microbes.⁷

Various natural substances are proposed in community to enhance the immune system.⁸⁻¹³ They are known as the immunostimulators, substances that augment, stimulate, activate, potentiate or modulate the immune response at either the cellular or humoral level.¹⁴ Obtained from the nature, however the use of herbs as

immunostimulant is based on empiric therapy. It has been delivered from one generation to another generation. In traditional medicine, it is considered that a specific herb gives the healing effect to a specific disease, or several diseases. This empiric-based therapy is not actually identified, whether the medicinal effect is due to the clinical symptom that declines, or the herbs substances are pharmacologically active to organs.

Biomedical experiment is expected to understand and clarify the various effects of herbs on immune system. In future, the practice of herbal medicine is expected not only as an empiric-based therapy, but is also evidenced in biomedical laboratory experiments. Clinical trials must be conducted before the distribution of the herbal medicine after proved by laboratory experiments.

There are a lot of studies about *Phaleria macrocarpa* and *Phyllanthus niruri*, which demonstrate their functions in enhancing immune system, body homeostasis, and inducing the cancer cells apoptosis.⁸⁻¹³

Phaleria macrocarpa is identified as an immunostimulator for the Natural Killers 1.1 (NK1.1 cells), by significantly increases killing activity via Interferon-gamma (IFN- γ) synthesis.⁸ IFN- γ is a potent activator of monocytes and macrophages to kill and ingest the microbes.⁹

Phyllanthus niruri extract roles as a herbal medicine via several pathways. An in-vitro study conducted on mice proved the extract increased the activities of Bone-Marrow-derived Dendritic cells, which resulted in T_H1 cells proliferation, followed IFN- γ secretion. The macrophages activity is augmented via this mechanism.¹⁰

Both of the herbs have the good potency in enhancing immune system, and there was not available research publication about the effects of combination of herbs extracts yet. Considering to this, it was required to do a research whether the combination treatment resulted in better immune response.

The research was purposed to evaluate the differences of BALB/c mice peritoneal macrophages activity on the combination treatment of *Phaleria macrocarpa* and *Phyllanthus niruri* extracts. The macrophages phagocytic activity was represented by phagocytic index and measured with Latex-Beads-Particle Methods (produced by Sigma-Aldrich Steinheim, Germany). It was expected that the combination of herbs extract resulted in better macrophages phagocytosis activity. The dosage for each extract was based on the previous researches conducted, for *Phaleria* 0,14mg/day, for *Phyllanthus* 0.4mg/day, and combination extract with the same dossage respectively.^{9, 13} Each of extract was administered in 0,5 mL water for every mouse. The treatment was given in 7 days, based on the previous researches significances. This research had not been conducted before therefore researcher proposed to do this research.

1.2. Research Questions

1. Is there any difference on Peritoneal Macrophages Phagocytic Index of BALB/c mice which are treated with combination extracts of *Phaleria macrocarpa* and *Phyllanthus niruri* compared to control?
2. Is there any difference on Peritoneal Macrophages Phagocytic Index of BALB/c mice which are treated with combination extracts of *Phaleria*

macrocarpa and *Phyllanthus niruri* compared to *Phaleria macrocarpa* single extract?

3. Is there any difference on Peritoneal Macrophages Phagocytic Index of BALB/c mice which are treated with the combination extracts of *Phaleria macrocarpa* and *Phyllanthus niruri* compared to *Phyllanthus niruri* single extract?

1.3. Research Aims

1. To measure the difference on Peritoneal Macrophages Phagocytic Index of BALB/c mice which were treated with the combination extracts of *Phaleria macrocarpa* and *Phyllanthus niruri* compared to control.
2. To measure the difference on Peritoneal Macrophages Phagocytic Index of BALB/c mice which are treated with the combination extracts of *Phaleria macrocarpa* and *Phyllanthus niruri* compared to *Phaleria macrocarpa* single extract.
3. To measure the difference on Peritoneal Macrophages Phagocytic Index of BALB/c mice which are treated with the combination extracts of *Phaleria macrocarpa* and *Phyllanthus niruri* compared to *Phyllanthus niruri* single extract.

1.4. Research Benefits

1. Discovering the role of combination extracts of *Phaleria macrocarpa* and *Phyllanthus niruri* on BALB/c mice Peritoneal Macrophages Phagocytic Index.
2. Discovering the role of single extract of *Phaleria macrocarpa* on BALB/c mice Peritoneal Macrophages Phagocytic Index.
3. Discovering the role of single extract of *Phyllanthus niruri* on BALB/c mice Peritoneal Macrophages Phagocytic Index.

1.5. Research Originality

Table 1. Research Originality

No	Articles	Methods	Results
1	Budijitno S. <i>Peran Interferon Gamma, Perforin, Granzim, Terhadap Apoptosis Sel Kanker Payudara Yang Mendapat Neoadjuvan Kemoterapi Dan Ekstrak Phaleria macrocarpa</i> [dissertation]. Semarang (Indonesia): Universitas Diponegoro; 2011. ⁹	Experimental research; Randomized pre and post test controlled group design; Human; Mahkota Dewa (<i>Phaleria macrocarpa</i>) Extract Effects in NK cells activity and breast cancer cells apoptosis.	<i>Phaleria macrocarpa</i> increased the therapeutic of invasive ductal breast cancer with Adriamycin via the increase of NK cells granzyme-perforins secretion, and apoptosis of cancer cells.
2	Maat S. <i>Phyllanthus niruri L sebagai immunostimulator pada mencit.</i> [dissertation]. Surabaya (Indonesia): Universitas Airlangga; 1996. ¹¹	Experimental research; Post test only control group design; mice; Meniran (<i>Phyllanthus niruri L.</i>) Extract; Immune System.	<i>Phyllanthus</i> extract increases the neutrophils phagocytosis function, NK cells cytotoxicity, T lymphocytes activity, secretion of cytokines.

3	Sawitri E. <i>Pengaruh Ekstrak Pn terhadap Kanker Kolon Tikus Spraguedawley Yang Diinduksi 1.2-dimetilhidrazin</i> [dissertation]. Semarang (Indonesia): Universitas Diponegoro; 2012. ¹³	Experimental research; Post test only control group design; Spraguedwaley rats; Meniran (<i>Phyllanthus niruri</i> L.) Extract; Immunologic status to colon cancer development.	The administration of <i>Phyllanthus</i> extract increased immunologic status through the increasing of lymphocytes infiltration and perforins expression, & inhibition the colon cancer development by increasing apoptotic index.
---	------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

The differences of this research with the previous studies were the independent variables and the experimental subjects. This study principally measured the effects of the combination of *Phaleria macrocarpa* and *Phyllanthus niruri* extracts on peritoneal macrophages phagocytic index of BALB/c mice with the Latex-Beads Particles Methods.