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Immunostimulant Activities of *Dioscorea esculenta* L. Tubers Based on Phagocytic Activity and Lymphocyte Proliferation In Vitro

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ABSTRACT

The immune system plays an important role for the body, especially in protecting it from exposure to bacteria, viruses, and other foreign bodies. Improving the immune system can be done by daily consumption of certain foods. Foods that can be developed into an immunostimulant are tubers, one of which comes from the genus Dioscorea. *Dioscorea esculenta* L., known as gembili tuber, is widely found in Indonesia, but has not been widely tested for its activity as an immunostimulant both against the innate and adaptive immune systems. This study aims to determine the immunostimulant activity of aqueous extract (AE), polysaccharide fraction (PF), and non-polysaccharide fraction (NPF) of gembili tubers against macrophage phagocytosis activity and lymphocyte proliferation. Test of phagocytosis activity and lymphocyte proliferation was performed in vitro by adding AE, PF, NPF gembili tubers 12.5, 50, and 100 µg/mL, inulin 100 µg/mL, and positive control 10 µg/mL as a comparison in macrophage cells and mouse lymphocyte cells. Phagocytosis activity was expressed in phagocytosis capacity and phagocytosis index, while lymphocyte proliferative activity was expressed in proliferative stimulating index. The results showed that AE, PF, and NPF could increase macrophage phagocytosis activity, with the highest activity observed at AE 100 µg/ml, PF 100 µg/ml, and NPF 12.5 µg/ml. AE, PF, and NPF were also shown to increase lymphocyte proliferation activity, with the most significant enhancement observed at AE 12.5 µg/mL, PF 12.5, and NPF 50 µg/mL.

Keywords: *Dioscorea esculenta* L.; lymphocyte; immunostimulant; macrophage.

INTRODUCTION

The body's immune system is crucial, particularly in protecting against infections, viruses, and other foreign substances. When the immune system is exposed to substances that are considered foreign, two types of immune responses will occur: innate immune responses and adaptive immune responses (Abbas et al., 2012). Innate immunity is a natural defense mechanism. This mechanism acts as the first line of defense and inhibitor of most pathogens before they become visible infections (McComb et al., 2019). Meanwhile, specific immunity (adaptive/acquired) arises as a result of certain antigen stimulation, as a result of the body having been exposed before (Abbas et al., 2012).

External compounds that act as immunostimulants can be used to enhance the immune response. Immunostimulant is an immunomodulator that can increase the body's resistance to various infections through increasing the basal rate of immune response (Jain et al., 2022). Improving the immune system can be done through daily food consumption. Some examples of foods that can be developed into an immunomodulator are tubers from the genus Dioscorea, which exists in Indonesia. *Dioscorea alata* L., water yam or greater yam locally known as *uwi*, has been proven as an immunostimulant.

Research on immunostimulant from *uwi* tubers including water extract was shown to stimulate humoral immune responses and also modulate phagocytosis and peritoneal macrophage-related activity, up-regulation of IL-4 and IL-10 levels, IgM and down-regulation of NO, IL-2, IFN-γ, TNF-α, PGE2, and COX activity (Dey et al., 2016). In addition, hot water extracts precipitated with 80% ethanol have been shown to

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contain inulin-like polysaccharide compounds (polysaccharide fractions) that can enhance serum IgA-mediated immunomodulation (Bandyopadhyay et al., 2021). Yam polysaccharides can significantly increase lymphocyte proliferation and NK cell activity in vivo, and can significantly increase the ability of mouse lymph cells for IL-2 production and peritoneal macrophages for TNF-α production (Huang et al., 2020). Water extract of *uwi* tubers has also been shown to contain a dioscorin protein that can stimulate several signaling molecules (NF-κB, ERK, JNK, and p-38) and induce expression of cytokines (TNF-α, IL-1β, and IL-6) in RAW 264.7 macrophage cells (Fu et al., 2006).

This study sought to explore the potential immunostimulant activity of tubers from other Dioscorea genus that are also found in Indonesia, namely *Dioscorea esculenta* L. or *gembili* tubers. *Gembili* bulbous plants grow in various parts of Indonesia, growing abundantly in people's yards and forests. Some local communities in Indonesia have long used *gembili* tubers as an alternative source of carbohydrates. *Gembili* tubers have long been traded in traditional markets. Despite this, its utilization remains limited (Silalahi, 2022; Sri Winarti et al., 2013).

Research on *gembili* tubers as immunostimulants has not been extensively conducted. Research on *gembili* tubers as immunostimulants conducted by Winarti et al. (2014) has shown that inulin in hot water extracts of *gembili* tubers that have been purified can increase the production of IL-6 and TNF-α both in J774.1 and P-Mac cells with a concentration of 8 μg/mL (S Winarti et al., 2014). Research also shows that *gembili* tubers have the potential as prebiotics (Khasanah et al., 2019; S Winarti et al., 2014). The prebiotic activity of *gembili* tubers can play a significant role in boosting the immune system through immunomodulation along the gastrointestinal tract, focused on gut-associated lymphoid tissue (GALT). One of the constituents of this tissue is Peyer's patches with follicles containing B and T lymphocytes (Vogt et al., 2015).

Gembili tubers exhibit immunostimulant and prebiotic activity due to the content of water-soluble polysaccharides (PLA or soluble dietary fiber). *Gembili* tubers exhibit significantly higher levels of inulin compared to *uwi* tubers, with values of 14.77% (S. Winarti et al., 2011), 67.66 mg (Zubaidah & Akhadiana, 2013), 23.21 % per gram extract (Martono et al., 2019), and 26.22% (Masrikhiyah & Fera, 2019). *Gembili* tubers in water extract were shown to contain glucomannan by 39.49% (Herlina, 2012; Sareu et al., 2021). *Gembili* tubers also have non-polysaccharide bioactive components such as dioscorine and diosgenin which can function as immunostimulants (Andriani et al., 2020; Prabowo et al., 2014).

Based on the above-mentioned background, this study aims to demonstrate the immunostimulant activity of *gembili* tubers on both the innate and adaptive immune systems. The research study focused on the immunostimulant effects of the aqueous extract (AE), polysaccharide fraction (PF), and non-polysaccharide fraction (NPF) of *gembili* tubers. This included testing macrophage phagocytosis activity and lymphocyte proliferation in vitro.

MATERIALS AND METHODS

Plant Materials

Fresh *Dioscorea esculenta* L. (*gembili*) tubers were obtained from Gundi Village, Ledokdawan Village, Geyer District, Grobogan Regency, Central Java. The tubers were chosen for their freshness and maturity, which were harvested 8-9 months after the planting period. *Dioscorea esculenta* L. plants were identified and authenticated in the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia (certificate no. 17.12.4/UN1/FFA.2/BF/PT/2023).

Animals

Male Balb/c mice (*Mus musculus*) aged 6-8 weeks old and weighing 20-30 g were obtained from the Animal Center of Pharmacology and Toxicology Laboratory, Sekolah Tinggi Ilmu Farmasi Yayasan Pharmasi Semarang. All animals were acclimatized for one week before experimentation. They were housed in an animal room with a temperature of (22 ± 3)^{o} C and humidity of 60% under a 12-h light/dark cycle. A standard pelleted basal diet and water were provided ad libitum. All experimental procedures were approved by the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada – DR. Sardjito General Hospital, Indonesia (certificate no. KE/FK/1667/EC/2023).

Sample Preparation

Gembili tubers were cleaned and washed under running water, peeled, and cut into small pieces, then blended with the addition of hot water at a temperature of 80^{o}-90^{o}C with a ratio of 1:4 (tubers: hot water). Next, it was filtered to take the filtrate and cooled. The filtrate obtained hereinafter is called the aqueous extract of *Dioscorea esculenta* L. (AE). This water extract was partially dried using a freeze-dryer

(Winarti et al., 2013). The aqueous extract of *gembili* tubers was further precipitated using 96% ethanol (40% of the total filtrate volume). This solution was stored in a temperature freezer of ± -10°C for 18 hours until a precipitate was obtained. It was then diluted (thawing) at 8°C for 2 hours and drawn with a vacuum pump to obtain a precipitate. The precipitate was dried using a freeze-dryer. The precipitate is hereinafter referred to as the polysaccharide fraction of *Dioscorea esculenta* L. (PF). Meanwhile, the filtrate was concentrated with a vacuum rotary evaporator at a temperature of 60°C for 6-7 hours. The viscous fraction was dried using a freeze dryer. This fraction is referred to as the non-polysaccharide fraction of *Dioscorea esculenta* L. (NPF) (Zubaidah & Akhadiana, 2013).

Phytochemical Analysis

Preliminary phytochemical screening of the fractions was carried out according to Indonesian Herbal Pharmacopoeia and Harborne (Harbone, 1998; Kemenkes Republik Indonesia, 2017).

Immunomodulatory Effect on Mouse Peritoneal Macrophage

After the mice were killed with a light anesthesia, the skin of the abdomen was opened aseptically. Macrophages were isolated from peritoneal fluid by injecting 10 mL of cold RPMI-1640 (Sigma Chem, St Louis, MO, USA). The animals were allowed to stand for 3 minutes until macrophages attached to the peritoneal cavity, and the area surrounding the intestine could be detached and suspended in the RPMI medium. Peritoneal fluid was removed from the peritoneal cavity and aspirated with a syringe. Peritoneal fluid was aspirated and centrifuged at 1200 rpm and 40°C for 10 minutes. The supernatant was removed, and 3 mL of complete RPMI medium containing fetal bovine serum was added to a density of 2.5×10^6 cells/mL. It was then cultured on 24-well microplates, which were covered by coverslips and incubated overnight at 37°C in 5% CO_2 incubator. Further, the medium was removed and washed once with RPMI. After washing with one mL of RPMI 1640 medium, various concentrations of AE, PF, NPF (12.5, 50, and 100 µg/mL), inulin 100 µg/mL, and positive control 10 µg/mL were added to each well at a volume of 100 µL. Culture plates were then incubated for one hour at 37°C. The latex beads with a diameter of 3.0 mm (Sigma Chem) were added in 24-well culture plates at 50 µL/well and incubated for one h at 37°C. After washing three times with phosphate buffer saline (PBS-GIBCO) using 1 mL/well, the wells were fixed with methanol and colored by Giemsa 20% v/v for 30 min, then washed with distilled water and dried at room temperature. The macrophage cells (100 cells) phagocytosing latex beads (in the coverslips) were counted using a light binocular microscope at 100× magnification. The activity of macrophage phagocytosis was evaluated using the phagocytic index and phagocytotic capacity (Nurrochmad et al., 2015).

$$\%\ phagocytotic\ capacity = \frac{\Sigma macrophages\ that\ phagocytosed\ latex\ beads}{100\ macrophages\ counted}\ x\ 100$$

$$Phagocytic\ index = \frac{\Sigma latex\ beads\ that\ were\ phagocytosed\ by\ 100\ macrophages}{\Sigma active\ macrophages}$$

Immunomodulatory Effect on Lymphocyte Proliferation

The spleens of mice were isolated aseptically, 10 mL of RPMI was pumped into the spleen until the lymphocyte cells suspension was completely transferred to a 50-mm petri dish, then the cell suspension was centrifuged at 2000 rpm and 4°C for 10 min. The supernatant was decanted, and the pellet was suspended in Tris-buffered NH_4Cl to lyse erythrocytes and left at room temperature for 5 min. Centrifugation was repeated for 10 min, and the supernatant was discarded. Pellets were suspended in complete RPMI medium to a density of 1.5 x 10^6 cells/mL, then cultured at 100 µL/well in 96-well microplates. Phytohemagglutinin (2 µL/well) was added to each well and incubated in a 5% CO_2 incubator at 37°C for 24 h. The AE, PF, NPF (12.5, 50, and 100 µg/mL), inulin 100 µg/mL, and positive control 12.5 µg/mL, and a media control were then added and incubated for 48 hours. Following this, 10 µL MTT [3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium-bromide] 5 mg/mL solution was added to each well, then incubated again under the same conditions for 4 h. Subsequently, 50 µL stop reagent (10% SDS) was added to each well. Lymphocyte cell proliferation was measured from the optical density with an ELISA microplate reader at 550 nm (Nurrochmad et al., 2015).

$$stimulation\ index = \frac{(OD\ sample - OD\ media)}{(OD\ cell - OD\ media)}$$

Statistical analysis

Data from all experiments were expressed as the mean ± standard error of the mean (SEM), and statistically significant differences between groups were analyzed by one-way analysis of variance or the Kruskal–Wallis test followed by the least significant difference (LSD) or Mann–Whitney test. Statistical analysis was performed

using SPSS version 23 (Armonk, NY: IBM Corp.), and (*) $p \leq 0.05$ was considered to indicate statistically significant differences.

RESULTS

Phytochemical Analysis

From the extraction and fractionation of *gembili* tubers, the yields for aqueous extract (AE), polysaccharide fraction (PF) and non-polysaccharide fraction (NPF) were 2.51%, 12.00%, and 2.75%, respectively. Phytochemical screening procedures were carried out to determine the potential active compounds that may be involved in the observed biological activities. The presence of secondary metabolites in AE, PF, and NPF was determined, and the results are summarized in Table I.

Table I. Preliminary phytochemical screening of AE, PF, and NPF

Test	AE	PF	NPF
Phenolic	-	-	-
Polyphenol	+	+	+
Alkaloid	-	-	+
Saponin	+	-	+
Tanin	-	-	-
Flavonoid	+	-	+
Steroid	-	-	-
Terpenoid	-	-	-

(+): present; (-): absent

Immunomodulatory Effect on Mouse Peritoneal Macrophage

Macrophage activity can be measured by its ability to phagocytose latex bead particles. This test uses the latex bead method as shown in Figure 1. Latex beads are microparticles that are comparable in size to bacteria but are almost the same in size and are degraded in the cellular environment. Moreover, the beads do not contain bacterial components that activate innate immune receptors (Ueno et al., 2021). The character of latex beads prohibits them from being painted. As a result, during the staining process with Giemsa, the macrophages will appear purplish, while the latex is colorless (Figure 1). The results of this staining can be used to differentiate between phagocytosed and non-phagocytosed latex (Karimaa, 2019).

The test results showed that in the % phagocytic capacity parameter, all concentrations of AE, PF, and NPF were significantly different from the solvent control (Na CMC). This suggests that AE, PF, and NPF *Dioscorea esculenta* L. could increase the number of macrophages that phagocytose latex particles (Figure 2). AE and PF showed an increase in phagocytic capacity as the concentration increased, and the highest activity was observed at a concentration of 100 μg/mL. Meanwhile, as NPF concentration increased, its phagocytic capacity decreased, and the highest activity was found at a concentration of 12.5 μg/mL.

The results of the phagocytosis index test showed that all concentrations of AE, PF, and NPF were significantly different from the control solvent (Na CMC). This indicates that AE, PF, and NPF *Dioscorea esculenta* L. could increase the number of latex particles phagocytosed by macrophages, except NPF 100 μg /mL (Figure 3). The phagocytosis index of AE, PF, and NPF displayed the same pattern, with a decrease in the number of latex particles phagocytosed by macrophages as the concentration increased. The highest activity was found in AE 12.5 μg/mL, PF 50 μg/mL, and NPF 12.5 μg/mL.

Immunomodulatory Effect on Lymphocyte Proliferation

The adaptive immune response can be observed through the proliferation activity of lymphocyte cells. Lymphocyte cells are immune cells that play a role in the adaptive immune response. The adaptive response is based primarily on specific antigen receptors expressed on the cell surface of lymphocytes (Chaplin, 2010; Radji, 2015). The immunomodulatory effect is demonstrated by the ability of lymphocytes to multiply when given extracts or fractions as antigens. Lymphocyte proliferation activity can be determined by assessing the lymphocyte proliferation stimulation index value as shown in Figure 4.

The results of the stimulation index at various concentrations of AE, PF, and NPF showed values of less than 2 (two). Gembili tuber AE, PF, and NPF were found to increase lymphocyte proliferation activity at different concentrations. AE 12.5 μg/mL, PF 12.5, and NPF 50 μg/mL had a significant stimulating effect ($P \leq 0.05$) on lymphocyte proliferation. Administration of positive control 12.5 μg/mL increased lymphocyte proliferation activity ($P \leq 0.05$), while administration of 100 μg/mL inulin did not have a significant stimulatory effect on lymphocyte proliferation ($P \geq 0.05$).

DISCUSSION

This research is a preliminary study that aims to determine the immunostimulatory activity of *gembili* tubers (*Dioscorea esculenta* L.) on both innate and adaptive immune responses. Bioactive compounds were withdrawn through three further stages which produced water extract (AE),

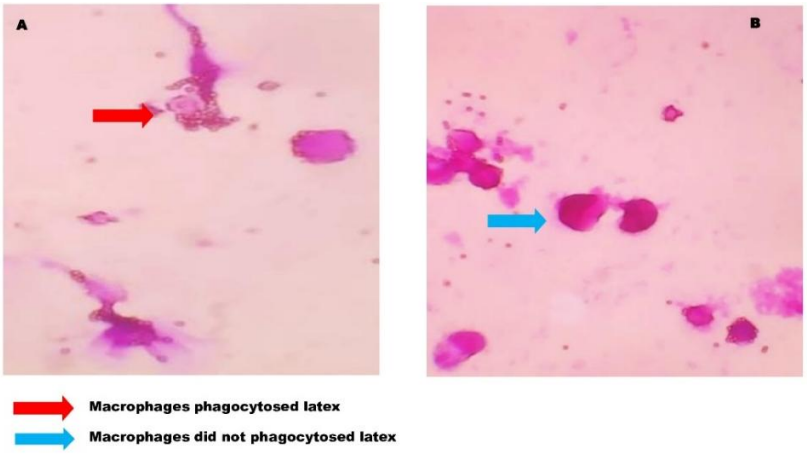

Figure 1. Phagocytosis activity of macrophages against latex. Macrophages stained by Giemsa 20%, ×100. The macrophage activity in the treatment group (A) and the control cell group (B)

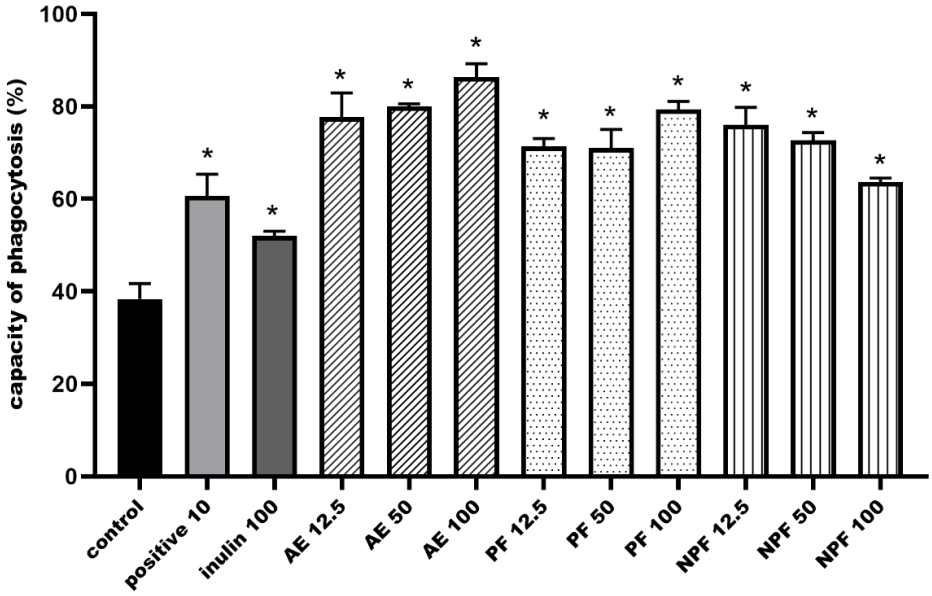

Figure 2. Effect of aqueous extract (AE) (A), polysaccharide fraction (PF) (B), and non-polysaccharide fraction (NPF) (C) *Dioscorea esculenta* L. on the phagocytic capacity of mouse peritoneal macrophages (mean ± SEM, n=3). *P ≤ 0.05 were significantly different compared with the control.

polysaccharide fraction (PF), and non-polysaccharide fraction (NPF). The innate immune response was observed from the phagocytic activity of macrophage cells. Macrophages are cells in tissues that originate from white blood cells called monocytes. Macrophages begin from monocytes found within the blood circulation, which mature and separate, and subsequently move to tissues. The main function of macrophages is to phagocytose outside particles such as microorganisms, macromolecules counting antigens and even cells or tissues themselves that are harmed or dead. Phagocytosis is a process that begins with the contact of a foreign particle with a cell surface receptor followed by the elongation of the plasma membrane surrounding the foreign particle, called the phagosome (Abbas et al., 2012).

Parameters observed included phagocytic capacity and phagocytic index of macrophage cells. Phagocytic capacity refers to the number of macrophages that phagocytose latex particles, and phagocytic index indicates the amount of latex phagocytosed by macrophages. In this study, the phagocytosis process was characterized by the process of latex being consumed by macrophages. Latex is a versatile system for in vitro and in vivo analysis of many phagosome functions (Ueno et al., 2021). Because latex is inert, it is eaten

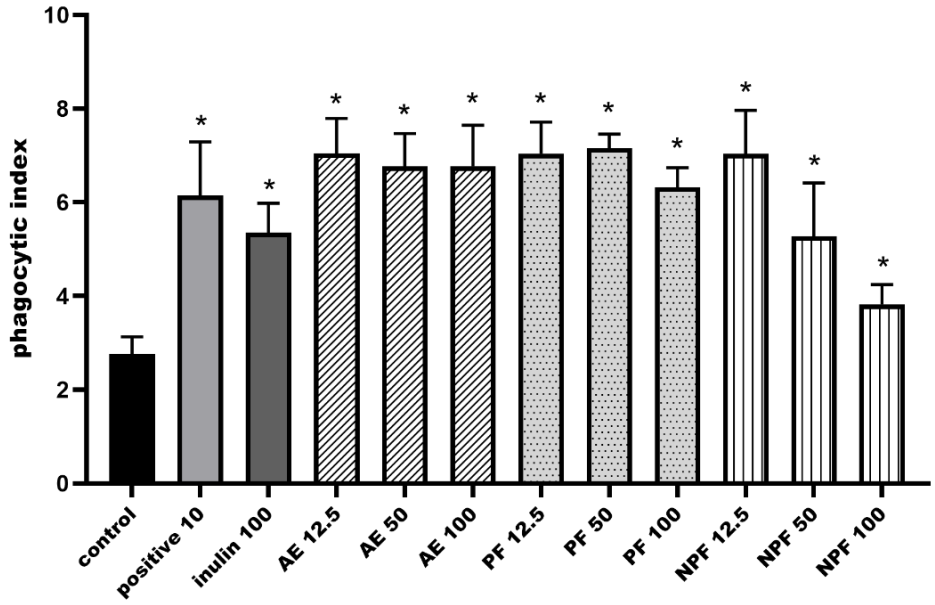

Figure 3. Effect of aqueous extract (AE) (A), polysaccharide fraction (PF) (B), and non-polysaccharide fraction (NPF) (C) of *Dioscorea esculenta* L. on the phagocytic index of mouse peritoneal macrophages (mean ± SEM, n=3). *P ≤ 0.05 were significantly different compared with the control.

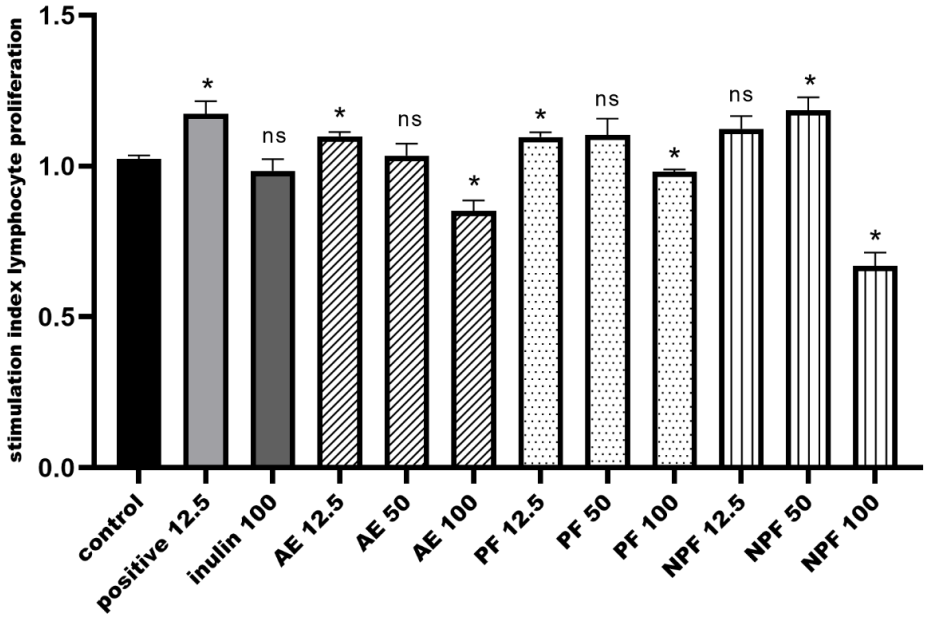

Figure 4. Effect of aqueous extract (AE) (a), polysaccharide fraction (PF) (b), and non-polysaccharide fraction (NPF) (c) *Dioscorea esculenta* L. on the proliferation of phytohemagglutinin-stimulated mouse lymphocytes (mean ± SEM, n=3). *P ≤ 0.05 was significantly different compared with the control.

by macrophages due to chemical mechanical contact (Karimaa, 2019).

Macrophages are isolated from the peritoneum cavity. The peritoneal cavity is the fluid-filled abdominal cavity and is a gathering place for immune cells such as macrophages, B cells, and T cells (Chaplin, 2010). The peritoneal fluid of mice was then stained with Giemsa paint. The macrophages turned to purple, while the latex particles were not stained with Giemsa paint (Figure 1). Increased macrophage phagocytic activity was characterized by an increase in the

number of macrophages that phagocytosed latex particles (phagocytosis capacity) and an increase in the number of latex particles phagocytosed by macrophages (phagocytosis index).

The adaptive immune response is observed from the proliferation activity of lymphocytes. The proliferation response in lymphocyte culture is used to describe lymphocyte function and the body's immune status. Lymphocyte cells were obtained from the spleen of mice because the highest number of lymphocyte cells (including T cells and B cells) was in the spleen (around 65%) compared to other tissues. Lymphocyte cells are found in the spleen tissue with varying numbers of lymphocytes 72 x 10^9 (Abbas et al., 2012). Proliferation in lymphocyte cells is a process of cell maturation and multiplication through cell division called mitosis to produce active effector cells that play a role in specific and non-specific immune responses for the elimination of pathogenic microorganisms and other foreign substances (Winanta et al., 2023). This immune response indicator can stimulate the work of the immune system in its task of maintaining the body's health. In the presence of antigens in the body, the ability of lymphocyte cells increases to produce antibodies against these antigens, and ultimately the body's resistance will improve.

The results of the immunostimulatory activity of macrophage phagocytosis showed that AE, PF, and NPF of *gembili* tubers significantly increased the phagocytotic capacity and phagocytotic index of macrophage cells compared with control cells ($p \leq 0.05$). AE, PF, and NPF exhibited higher macrophage phagocytic activity than positive control and inulin. Meanwhile, the results of the immunostimulatory activity of lymphocyte proliferation showed that AE, PF, and NPF at certain concentrations could increase lymphocyte proliferation, namely AE 12.5 µg/mL, PF 12.5, and NPF 50 µg/mL. The immunostimulatory activity against lymphocyte proliferation in the positive control group was higher compared to the AE and PF groups, but lower than the NPF group. The inulin group did not have a significant effect on lymphocyte proliferation ($p \geq 0.05$).

In this study, positive controls used inulin and a commercial product containing 250 mg of *Echinacea purpurea*. *E. purpurea* is reported to have the ability to increase phagocytosis because of the content of polysaccharides that can activate macrophage cells and NK cells and have been tested preclinically and clinically as immunostimulants (Gajewski et al., 2013). Inulin is a carbohydrate or polysaccharide with a chain length of 2-60 units, called fructan and is a fiber that can dissolve in water, but cannot be digested by digestive enzymes (Dewanti & Rahayuni, 2012). Inulin regulates the immune system through several pathways. Inulin plays a role in IgA secretion, suppressing the NF-κB, ERK ½, and JNK pathways, thereby reducing pro-inflammatory factors (IL-6, IL-12, p40, TNF-α) through stimulation of lymphocytes and dendritic cells and increasing immune effects through activation of the complement system (Tawfick et al., 2022). Inulin also activates immunological cells in Peyer's patches, including IL-10 production and cytotoxic NK cells (Gualtieri et al., 2013).

The immunostimulatory activity of AE, PF, and NPF of *gembili* tubers both on macrophage phagocytosis and lymphocyte proliferation was due to the active compound content in them. The results of the phytochemical analysis in this study (Table 1) showed that *gembili* tuber AE contained compounds from the polyphenols, flavonoid, and saponin groups. Gembili tuber PF contained compounds from the polyphenols group, while *gembili* tuber NPF contained compounds from the polyphenols, alkaloid, saponin, and flavonoid groups.

AE contains polyphenols, flavonoids, and saponin, because water is considered a better solvent than others when extracting phenolic compounds mostly because, in previous works, aqueous extracts showed higher efficiency (Sarikurkcu et al., 2020). Furthermore, AE is precipitated by 96% ethanol, so PF and NPF are obtained. PF and NPF also contain polyphenols, as ethanol is a good solvent for the extraction of polyphenols (Nakilcioglu-tas & Otles, 2021; Nguyen et al., 2020). Ethanol is also used to precipitate polysaccharides (PF), one of which is inulin (Murwindra, 2019). NPF contains alkaloids, saponins, and flavonoids, because the solvent used to obtain NPF is ethanol. Ethanol is a solvent with high solubility (dielectric constant 24), so it is useful for dissolving all substances, both polar and semipolar (Puspitasari et al., 2023). Alkaloid compounds, flavonoids, and saponins are polar and generally can be attracted by polar solvents such as ethanol (Pusvitasari et al., 2021). AE, PF, and NPF do not contain steroids and terpenoids, as steroids, and terpenoid compounds are non-polar and can be dissolved by non-polar solvents such as n-hexane (Pusvitasari et al., 2021).

Several studies have shown the active compound content of *gembili* tubers. These include total saponin 17.65 mg/g (consisting of dioscin 4.35 mg/g, gracillin 7.75 mg/g, protodioscin 0.37 mg/g, protogracillin 1.97 mg/g, and saponin 9.22 mg/g), alkaloids 1.89 mg/100 g, flavonoids 12.4 mg/100 g, and saponin 20.01 mg/100g

(Lebot et al., 2019; Wang et al., 2023). These compounds have the potential to act as immunomodulators.

Saponins possess the capacity to effectively regulate both innate and adaptive immune responses. Plant saponins can promote the growth and development of the body's immune organs through a variety of signaling pathways, regulate the activity of a variety of immune cells, and increase the secretion of immune-related cytokines and antigen-specific antibodies, thereby exerting the role of immune activity (Shen et al., 2024). Furthermore, saponins are capable of stimulating the mammalian immune system by activating the innate immune response and promoting the generation of cytotoxic T lymphocytes (CTLs) that target exogenous antigens. Saponins exert their adjuvant activity via the immunostimulatory effects and by activation of cytokine production, e.g. interferons and interleukins (Sharma et al., 2020). Flavonoids have been widely known as immunomodulators. They have the potential to work against the lymphokines produced by T cells, thereby stimulating phagocytes to exhibit phagocytosis responses (Yuliastri et al., 2021). It is thought that alkaloids can also act as immunostimulants by increasing the production of IL-2 (interleukin 2) and lymphocyte proliferation in culture. Activated lymphocyte cells will activate cytokines such as interleukin-2 (IL-2) and interferon-γ (IFN-γ). These cytokines will activate macrophages to respond to antigens in the body. However, alkaloids can also be cytotoxic which can cause immunosuppressant activity (Karimaa, 2019).

CONCLUSIONS

The results showed that AE, PF, and NPF of *gembili* tubers (*Dioscorea esculenta* L.) were demonstrated to increase the number of macrophages that phagocytosed latex particles, the number of latex particles phagocytosed by macrophages, and lymphocyte proliferation activity.

AUTHOR CONTRIBUTION

IP was involved in plant collection, processing, and carrying out the experimental work. AN, NF, and MI supervised the overall study. IP contributed to the conceptualization and writing. AN, NF, and MI reviewed and edited the manuscript. All authors read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no financial or other conflicts of interest.

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