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Research Article

Immunomodulatory Activity of SNEDDS (Self Nano-Emulsifying Drug Delivery System) Alang-Alang Roots Extract (*Imperata cylindrica* (L.) P.Beauv.)

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ABSTRACT

The root of alang-alang (*Imperata cylindrica* (L.) P.Beauv.) is an herbal plant with various pharmacological activities. Dayak people in Central Kalimantan, Indonesia, believe a decoction of alang-alang roots can increase immunity. The development of nanoparticles is the right solution to increase absorption and drug potential. One of the methods in manufacturing nanoparticles is using Self Nano-Emulsifying Drug Delivery System (SNEDDS). The study aimed to know the potential of alang-alang roots as immunomodulatory agents formulated in the form of SNEDDS. Alang-alang roots were extracted using the maceration method using 70% ethanol solvent. Furthermore, the ethanol extract of alang-alang root was identified using Gas Chromatography-Mass Spectrometry (GC-MS). SNEDDS test of alang-alang root extract included transmittance test, emulsification time test, nanoemulsion droplet size test, Polydispersity Index (PDI), pH test, viscosity test, centrifugation test, nanoemulsion stability test, and immunomodulatory activity test. An immunomodulatory activity test was conducted *in vitro* on macrophage phagocytosis activity and lymphocyte proliferation. The results of compound identification using GC-MS showed 82 peak compounds, with the most significant percentage of compounds being cis-13-Octadecenoic (oleic acid), which amounted to 22.73%, and 5-Hydroxymethylfurfural at 17.38%. The SNEEDS formula test results showed that the SNEDDS formulation of alang-alang root extract obtained transmittance results with a percentage of >90%, emulsification time <1 minute, droplet size <100 nm, particle distribution Formula 1 in the polydisperse category while Formula 2 and Formula 3 in the monodispersion category, pH in the range of 5-6, viscosity in the range of 422.4 - 447.6 cP and stable test results without any phase separation and physical condition changes. SNEDDS administration of alang-alang root extract has immunostimulant activity on macrophage phagocytosis activity and lymphocyte cell proliferation. Therefore, the formulation of alang-alang root SNEDDS has the potential to immunomodulator activity.

Keywords:
Alang-alang root, SNEDDS, immunomodulator, *in vitro*

1. INTRODUCTION

Every human being has been designed to have a natural immune system in their body. The immune system is the main barrier to entering foreign objects or substances into the body. Many factors cause the decrease and increase in the body's ability to work. In addition, Indonesia, one of the countries with a tropical climate, is very suitable for the growth of various bacteria, viruses, and other disease agents[1].

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Therefore, pharmaceutical preparation is needed to modify the body's defense mechanism and overcome this. Immunomodulators that utilize natural ingredients can improve the body's immune system. Using natural materials with the potential as immunomodulators has become a new idea in conventional therapy to maintain the body's hemostasis[2,3]. Based on their effects, immunomodulator categories are divided into three, namely immunorestoration, immunosuppression, and immunostimulant[4]. Immunomodulators can be used in patients with immunodeficiency, cancer, and chronic infections. In AIDS and cancer patients, immunomodulators are used to prevent CD4+ cell damage[5].

One of the natural products that can often be utilized and has potential as an immunomodulator is an alang-alang root (*Imperata cylindrica* (L.) P.Beauv.). Alang-alang root is an herbal plant often used by the Dayak tribe in Kalimantan and several other regions in Indonesia. Dayak people in Kalimantan believe that alang-alang roots can improve the immune system. The alang-alang root contains alkaloids at 1.07%, and flavonoids at 4.8%. One of the compounds that has the prospect of increasing the activity of the immune system is the flavonoid group, which is rich in antioxidants. Other research shows that the largest content in alang-alang roots is a fatty acid compound of 15%[6]. Other studies also show the abundance of fatty acid compounds in alang-alang roots[7]. Fatty acids are essential in modulating the body's immune response, affecting effector function by altering membrane composition and fluidity by acting through adaptive and specific receptors.

The use of alang-alang root still needs to be improved, and there are shortcomings because it relies only on simple extracts and oral preparations. The development of nanoparticles is the right solution because they can increase absorption and drug potential. One nanoparticle-making method uses the Self Nano-Emulsifying Drug Delivery System (SNEDDS). SNEDDS systems have nanoemulsion droplet sizes that are less than 100 nm. This system increases the bioavailability of active substances in the body by facilitating the formation of solubilized droplets and increasing transport through the intestinal lymphatic system to increase the bioavailability of active substances and absorption in the gastrointestinal tract[8].

So, as a research development effort, an investigation will be carried out regarding the potential for enhancing the immune system by using SNEDDS of alang-alang roots (*Imperata cylindrica* (L.) P.Beauv.). This research is conducted to obtain empirical evidence regarding the benefits of alang-alang root SNEDDS as an immunomodulator. Hopefully, this research can provide further information about the benefits of alang-alang roots to the community.

2. MATERIALS AND METHODS

2.1. Materials

Alang-alang *(Imperata cylindrica* (L.) P.Beauv.) roots from Sampit, East Kotawaringin, Central Kalimantan, Indonesia were determined with No. 327/Lab. Bio/B/VII/2023, 70% ethanol, Methanol, aquadest, tween 20, PEG 400, VCO (Virgin Coconut Oil), Rosewell Park Memorial Institute (RPMI) 1640 medium (Gibco), RPMI 1640 complete medium containing 10% FBS (Fetal Bovine Serum) (Caisson), PBS (Phosphate Buffer Saline) (Gibco), latex beads (Sigma) 3 µm diameter, Giemsa paint, chloroform, round cover slips, 24-well microplate (Iwaki), petri dish, 96-well multiwell plate (Nunc, UK), AGF (Artificial Gastric Fluid) and AIF (Artificial Gastric Fluid) media, Tris-Buffered Ammonium Chloride.

2.2. Extraxtion

The preparation of alang-alang root extract (*Imperata cylindrica* (L.) P.Beauv.) was carried out using the maceration method in a ratio of 1:7 using 70% ethanol solvent. The solution was stirred and stored in a place protected from direct sunlight for five days and remacerated for three days. The solution was then filtered. The formed macerate was then concentrated using a rotatory evaporator until a thick extract was obtained.

2.3. Analysis of Compound Using GC-MS

Compound content analysis of alang-alang root extract (*Imperata cylindrica* (L.) P.Beauv.) using GC-MS method with ISQD1702517_1 instrument. The sample was dissolved with 1 ml ethanol, added solvent in a microtube, vortexed until homogeneous, then centrifuged at 9,500 rpm for 3 minutes. Take the supernatant; then, the sample solution is ready for injection. The column used is type HP-5MS UI as the stationary phase at a 325/350 ^{o}C temperature. The mobile phase used a Helium UHP (He) injector temperature of 230^{0}C with a split flow speed of 50ml/min—analysis run time for 32 minutes. Content analysis using GC-MS produces several bioactive compounds that can be seen from the peak chromatogram as identification of chromatographic and mass spectrometry data seen from the mass spectrum with each molecular weight of bioactive compounds[9].

2.4. SNEDDS Alang-alang Root Formulation

The SNEDDS preparation formula consists of alang-alang root extract, VCO as oil phase, Tween 80 as surfactant, and PEG 400 as cosurfactant. The following formula was made alang-alang root extract SNEDDS.

Mix 1 ml VCO oil phase with alang-alang root extract for 10 minutes in a beaker glass on a magnetic stirrer.Tween 80 surfactant was added slowly and homogenized with a magnetic stirrer for 10 minutes. Slowly add PEG 400 as a cosurfactant and stir for 30 minutes. The homogenized formula was conditioned in a waterbath at 40°C for 10 minutes. Next, sonication was carried out for 15 minutes at 40°C[10,11].

Table 1. SNEDDS formula.

Ingridient	Formula 1	Formula 2	Formula 3
Tween 80	8 ml	8 ml	8 ml
PEG 400	1 ml	1 ml	1 ml
VCO	1 ml	1 ml	1 ml
Alang-alang root extract	150 mg	200 mg	250 mg

2.5. The SNEDDS Characteristics

2.5.1. Transmittance

100 µL of SNEDDS of alang-alang root extract was taken and dissolved in 5 mL of distilled water in a test tube, stirred with a vortex for one minute, then tested using a UV-VIS spectrophotometer using distilled water as a blank at a wavelength of 650 nm[12].

2.5.2. Emulsification Time

Emulsification time was observed with SNEDDS of the alang-alang root extract in AGF, AIF, and distilled water media. The 100 ml media was conditioned at 37°C on a magnetic stirrer at 120 rpm. SNEDDS containing 1 ml of alang-alang root extract was dripped into the media quickly with the help of a syringe. Nanoemulsion was formed, characterized by the complete dissolution of SNEDDS in the media[13].

2.5.3. Particle Size and Polydispersity Index (PDI)

Particle size observation was carried out using a particle size analyzer (PSA) with Dynamic Light Scattering type (Microtrac S3500). SNEDDS of alang-alang root extract was dissolved into distilled water in a ratio of 1:100 and then tested using the PSA tool[13].

2.5.4. Viscosity Assay

Viscosity was measured using a Brookfield DV-1 viscometer at room temperature 25^{o}C 100 rpm. SNEDDS were put into a 100 ml beaker glass filled to the brim. The spindle speed was adjusted gradually from low speed to high speed, then from high speed to low speed.

2.5.5. pH

Testing the pH of the SNEDDS formulation of alang-alang root extract was carried out using a pH meter by dipping the pH meter into the SNEDDS preparation of alang-alang root extract.

2.5.6. Nanoemulsion Stability Assay

The nanoemulsion was prepared by taking 1 ml of SNEDDS of alang-alang root extract and adding up to 5 ml of the media. The media included distilled water, AGF media, and AIF media. The nanoemulsion mixture was homogenized with a vortex for 30 minutes. Condition at 37°C is the physiological temperature of the body. Observe every hour for 4 hours, including any changes in stability, such as phase separation and turbidity[14].

2.6. *In Vitro* Immunomodulator Assay

2.6.1 Macrophage Phagocytosis Activity

Macrophage cells will be from the peritoneal of male Balb/C mice. As much as 10 ml of RPMI media was injected into the peritoneum cavity. RPMI liquid in the peritoneum cavity is then taken back, put into a conical tube, and centrifuged. The supernatant was discarded, and 3 ml of RPMI liquid was added. Suspensions were carried out using complete RPMI media. The suspension was transferred as much as 200 µl of each well into a six-well plate with a coverslip. Incubated for 30 minutes at 37°C, then rinsed with complete media three times and incubated for 2 hours. The media was washed using RPMI liquid twice. Add 1 ml of complete RPMI liquid to each well and incubate again for 24 hours. A suspension of latex beads was made in PBS. Macrophage cells that have been incubated for 24 hours, taken with a drop pipette, and then washed two times with RPMI[15].

Macrophage cells cultured for 24 hours are taken and washed using RPMI 1640 medium. Then, add as much test material as the sample concentration series. They were then incubated in a 5% CO_2 incubator at 37°C for 4 hours. The test material was taken with a pipette and washed with RPMI-1640 medium. Latex bead suspension for each well was inserted at 200 µL and then incubated with a CO_2 incubator for 1 hour at 37°C so that macrophages could phagocytose the latex. Furthermore, washing was done three times using PBS so that particles

that were not phagocytosed were lost. Then, it was dried at room temperature and fixed for 30 seconds with methanol. Discard the methanol and let the coverslip dry. Giemsa 20% was dripped for 20 minutes, rinsed with distilled water, then dried at room temperature. Coverslips were observed using a light microscope at 400x magnification to see the number of macrophages phagocytosing latex[15].

Phagocytosis Index (PI) =

$$\frac{\text{Number of phagocytosed latex}}{\text{Number of activated macrophages (100)}}$$

Phagocytic Capacity (PC) =

$$\frac{\text{Number of macrophages that phagocytosed}}{\text{Number of macrophages counted(100)}} \times 100\%$$

2.6.2. Lymphocyte Proliferation

Lymph organs were removed and placed on a petri dish containing PBS. The spleen was washed three times using a PBS solution. Pump 10 ml of RPMI into the spleen until the suspension is obtained. The suspension was transferred to a 15 ml centrifuge tube to be centrifuged for 10 minutes at 1200 rpm. The pellet obtained was suspended in 1 ml of ammonium chloride buffer to lyse the erythrocytes. Mix the cells until homogeneous and centrifuged for 5 minutes at 1200 rpm. The supernatant was discarded, lymphocyte cells were suspended with 1 ml complete RPMI and then resuspended using RPMI[15]. The lymphocyte proliferation assay was performed using the MTT Assay method. Test samples were then added and incubated for 24 hours. After 24 hours of incubation, MTT [3-(4,5- 27 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide] was added to each well, incubation was carried out at 37°C for 4 hours. Next, a stopper reagent (10% SDS) was added to each well. After that, the absorbance was read at 550 nm using an ELISA Reader[15]

3. RESULTS AND DISCUSSION

3.1. Extraction

The extract obtained amounted to 213.792 grams. The characteristics of the alang-alang root extract are that it has a dark brown color and a distinctive smell and is very thick with consistency at room temperature, like very thick honey. After that, the yield calculation was carried out, and the yield obtained was 23.75%. This yield calculation is carried out to determine the percentage of the remaining sample amount from the maceration process and the effectiveness of the process that has been carried out.

3.2. GC-MS Compound Analysis

The results of compound identification using the GC-MS method obtained 82 peaks contained in ethanol extracts of alang-alang roots (Table 2). Analysis of compound content using the GC-MS method is a commonly used analytical technique to identify various contents contained in plant extracts. One of the main advantages of analysis using GC-MS is its high sensitivity and specificity. The following are some of the compounds identified with the most significant percentage and are assumed to be compounds with potential biological activity and activity for the body and the immune system.

Table 2. Chemical compounds with the largest percentage.

Peak	RT	Chemical Coumpounds	Molecular Formula	Molecular Weight	Ret. Area %
25	9.57	Catechol	$C_6H_6O_2$	110	6.15
27	9.97	5-Hydroxymethylfurfural	$C_6H_6O_2$	126	17.38
63	17.98	n-Hexadecadienoic acid	$C_{16}H_{32}O_2$	256	4.22
68	19.12	9,12- Octadecadienoic acid (Z,Z), methyl ester	$C_{19}H_{34}O_2$	294	2.57
70	19.73	cis-13 -Octadecadienoic acid	$C_{18}H_{34}O_2$	282	22.73
71	19.58	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.81

Catechol can also be used as a marker compound for developing anti-inflammatory drugs targeting phospholipase A2 (PLA2)[16]. The catalytic activity of PLA2 leads to the formation of inflammatory mediators such as leukotrienes, prostaglandins, and thromboxane.

5-Hydroxymethylfurfural, or 5-HMF, is a fraction of fructose and sucrose widely found in honey content[17]. In an *in vivo* study, it was found that 5-HMF can increase the production of IgG and IFN-γ[18]. In addition, the results showed that 5-HMF exhibited higher antiproliferative activity in human A375 melanoma cells compared to other cells. Another study also stated that 5-sulfoxymethylfurfural is efficacious as an antioxidant, antiallergic, anti-inflammatory, antibacterial, antiproliferative, and antihypoxic[19].

n-Hexadecadienoic acid is a compound from the fatty acid class; this compound has another name, palmitic acid. Palmitic acid has immunomodulatory activity and can regulate inflammatory processes and innate immunity, including monocytes, macrophages, and neutrophils. Previous research stated that palmitic acid is a ligand for TLR4 and induces TLR4-dependent

inflammatory cytokine production[20]. Hexadecadienoic acid has several biological activities, such as antioxidant and hypocholesterolemic[21]. 9,12- Octadecadienoic acid (Z,Z), methyl ester has antioxidant activity[22].

The alang-alang root extract tested using GC-MS cis-13-Octadecenoic got the most significant percentage area of 22.73%. cis-13-Octadecenoic acid, which has the chemical formula $C_{18}H_{34}O_2$, is a monounsaturated fatty acid also known as oleic acid. The compound cis-13-Octadecenoic acid is reported to have anti-inflammatory, cancer prevention, and hepatoprotective[23] and antioxidant activities[24]. In cell phagocytosis, oleic acid increases phagocytosis in neutrophil cells and proliferation in lymphocyte cells. In macrophage cells, oleic acid exerts an anti-inflammatory effect by inducing IL-1[24].

3.3. SNEDDS Characteristics

VCO is used as the oil phase because it has the advantage of being safe for peroral consumption and has higher saturated fatty acids than other vegetable oils. Triglycerides contained in VCO will more easily form emulsions, so they are commonly used for making SNEDDS[25]. The surfactant used is tween 80, nonionic, and not easily affected by electrolyte, acid, and base conditions. It remains stable in maintaining the layer between oil and water[10]. The cosurfactant used is PEG 400, a midchain hydrocarbon that plays a role in forming hydrogen chains that will increase spontaneity in forming nanoemulsion droplets[25]

Table 3. Transmittance and emulsification time analysis.

Formula	Sightings	Transmittance (%)	Emulsification Time (sec)		
			Aquadest	AIF	AGF
F1 (150mg)	Clear	99.99 ± 0.14	41.35	37.96	36.03
F2 (200mg)	Clear	99.18± 0.19	39.84	35.43	39.06
F3 (250mg)	Clear	98.95± 0.06	40.36	39.88	43.66

The transmittance test was conducted to determine the solubility of the sample. The percent transmittance value also shows the emulsification ability of a surfactant[25]. SNEDDS of alang-alang root extract from the three formulas with replication three times obtained a good transmittance value (close to 100%), which can be seen in (Table 3).

Emulsification time describes the ability of SNEDDS to emulsify in the body in the gastrointestinal tract. A good SNEDDS character can be emulsified by producing a transparent visual and can be completely emulsified in <1 minute and does not experience separation[26]. It can be seen from Table 3 that all SNEDDS formulas of alang-alang root extract has met the criteria of having an emulsification time of less than 1 minute. If you get a result < 1 minute, it indicates that SNEDDS has an emulsion system that can directly contact gastric fluid[27].

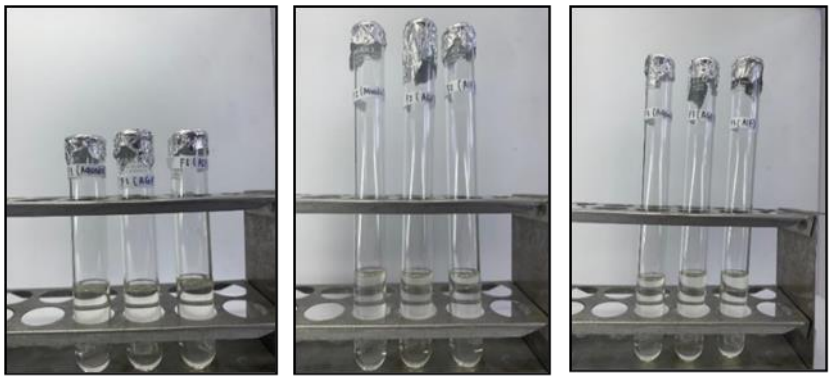
Table 4. Particle size, PDI results, pH and viscosity assay.

Formula	Size (nm)	PDI	pH	Viscosity (cp)
F1 (150mg)	10.78	1.346	6.03 ± 0.21	422.4
F2 (200mg)	11.63	0.480	6.03 ± 0.15	434.4
F3 (250mg)	10.97	0.052	6.10 ± 0.17	447.6

The nanoemulsion droplet size characteristic of SNEDDS should be <100 nm[28]. From the results of this study, the three formulas met the droplet size requirements of SNEDDS nanoemulsion. Factors that affect particle size include the effect of surfactants and homogenization methods[26]. The method used to maximize homogenization and particle formation is sonication. Sonication in the formation of nano-sized formula materials is very effective. The ultrasonic waves generated can break particles into smaller sizes.

Particle size diameter distribution is measured in terms of polydispersity index. The polydispersity index describes the uniformity of droplet size[29]. There are two categories of PDI, namely monodispersion and polydispersion. Monodispersion is in the range of 0.01-0.6, while polydispersion is >0.6. Based on the polydispersity index value obtained in the SNEDDS measurement of alang-alang root extract, F1 was found to be in the polydispersion category, while F2 and F3 were in the monodispersion category. Formulas F2 and F3 have more stable and uniform PDI values compared to F1.

The pH test was conducted to determine the acidity of the SNEDDS of alang-alang root extract. The pH test can show the stability of phase separation. Based on previous research, SNEDDS with a pH value of 4.5-6 does not occur phase separation[14]. pH values suitable for oral preparations are in the pH 5-7 range[25]. The results of this study show that the SNEDDS formulation of alang-alang root extract has a pH of 5-6 (Table 4). Based on the test results in this study, the viscosity of SNEDDS of alang-alang root extract was obtained between 422.4 - 447.6 cp (Table 4). These results show that the obtained alang-alang root extract SNEDDS formula will be easily poured into the capsule shell because it has viscosity characteristics that are less than 10,000 cp[30].



(a) (b) (c)

Figure 1. Nanoemulsion stability after 4 hours (a) F1 (b) F2 (c) F3 with media order from left aquadest, AGF media, and AIF.

The test results in Figure 1 show that the SNEDDS of alang-alang root extract are stable in each medium characterized by clear test samples, and no phase separation occurs in environments with alkaline pH, acidic pH, or the influence of electrolyte fluids in the gastrointestinal tract

3.4. *In Vitro* Immunomodulator Assay

3.4.1. Macrophage Phagocytosis Activity

Macrophage phagocytosis is the number of macrophage cells that actively phagocytose in 100 cells. The test animals in this study used BALB/c mice because they are more sensitive to antigen stimuli[31].

The results of testing macrophage phagocytosis activity can be seen in Figures 2 and 3. SNEDDS of alang-alang root extract, which has the highest phagocytosis index (PI) and phagocytosis capacity, is found in F3. The SNEDDS treatment of alang-alang root extract from the highest to the lowest concentration has a higher value than that of the cell control. The PI value = <1.2 has no immunomodulatory or immunosuppressive effect, PI = 1.3- 1.5 has a moderate immunomodulatory effect, and PI = >1.5 has a strong immunomodulatory effect[32]. The SNEDDS administration of alang-alang root extract can increase the phagocytosis ability of macrophage cells with the acquisition of PI> 1.5, which means it shows strong immunomodulatory properties. Phagocytosis capacity in F2 and F3 showed a more significant percentage of phagocytosis activity, significantly different from that of the control cells. These results indicate that the increase in concentration can increase the phagocytic activity of macrophage cells.

Previous research on mice treated with alang-alang root decoction found that the number of peritoneal macrophages, percentage of phagocytosis, and PI increased significantly [33]. Macrophages are part of the innate immune cells that mediate the body's nonspecific cellular immune response, which directs direct phagocytosis and indirect cytokine-mediated secretion. This suggests that alang-alang root extract can enhance nonspecific immunity in the body's immune system on macrophage phagocytosis activity.

Based on GC-MS analysis, alang-alang root extract contains 5-Hydroxymethylfultural or 5-HMF, which plays a role in the innate immune system. Administration of 5-HMF to rat test animals showed an increase in the antiviral innate immune system and serum IFN-β levels. Thus, 5-HMF can be seen to have a positive effect in increasing type I IFN production. Mechanistically, 5-HMF also increases macrophage RIG-I expression[34]. The RIG-1 receptor found on macrophages is essential in protecting cells from exposure to infection[35]. In addition, several ingredients that have functions as antioxidants contained in alang-alang roots also play a role in the body's immune function. Antioxidants can block the effects of reactive oxygen species (ROS) that can interfere with immunity and pro-inflammation[36]. The active compound solasonine ($C_{45}H_{73}NO_{16}$) contained in alang-alang roots also has immunomodulatory activity in the pinocytic activity assay test *in vitro*. In the immune system, pinocyte activity is the primary function of macrophage performance. The greater the pinocyte activity, the greater the phagocytic activity of macrophages[37].

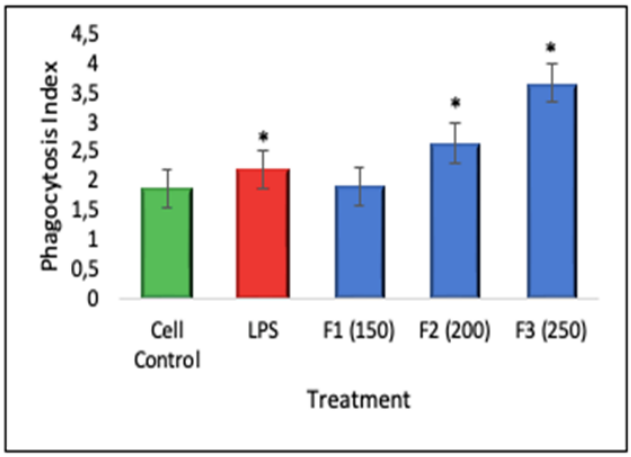


Figure 2. Macrophage phagocytosis index due to SNEDDS administration of alang-alang root extract. (mean±SD, n=3, α=0.05). (*) indicates a significant difference (P<0.05) between treatment, lipopolysacharide (LPS), and cell control.

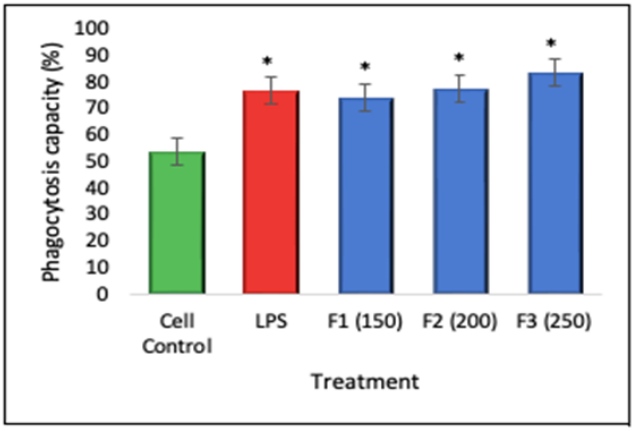


Figure 3. Phagocytosis capacity profile of macrophages due to SNEDDS administration of alang-alang root extract. Phagocytosis capacity (%) with 100x magnification (mean±SD, n=3, α=0.05). (*) indicates a significant difference (P<0.05) between treatment, lipopolysaccharide (LPS), and cell control.

3.4.2. Lymphocyte Proliferation Assay

Lymphocyte cells illustrate specific immune responses consisting of cellular and humoral responses. T lymphocytes mediate the cellular immune response, while the humoral response is initiated by B lymphocytes. Lymphocyte proliferation is a fundamental biological function of lymphocytes, namely the differentiation and cell division process. The method used in this study is the MTT assay method. The principle of the test method using MTT assay is based on the change of MTT, which is yellow into formazan in mitochondria that live in cells, which then turn purple.

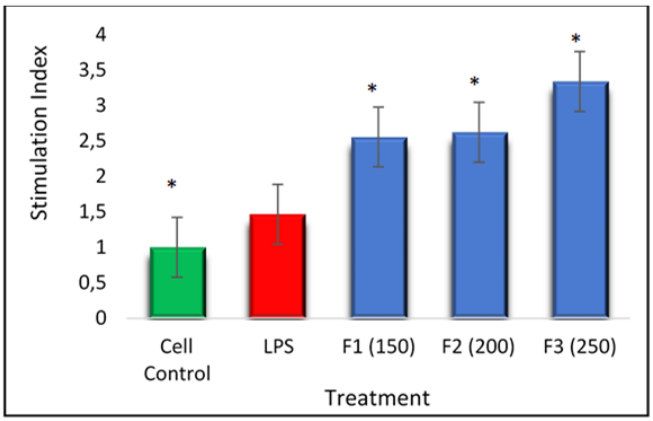


Figure 4. Lymphocyte proliferation stimulation index result profile (mean±SD, n=3, α=0.05).*shows a significant difference (P<0.05) between the treatment, lipopolysaccharide (LPS), and control groups.

The results of this study (Figure 4) show that alang-alang root extract can increase lymphocyte proliferation activity, as indicated by an increase in the lymphocyte proliferation stimulation index (SI) of several levels compared to cell control and positive control. The proliferation stimulation index parameter if the acquisition value of SI 2-3 = weak and can be positive if it has an SI value> 3, especially if more than one concentration is obtained[38]. Based on these parameters, this study shows that the administration of alang-alang root SNEDDS can affect lymphocyte proliferation. SNEDDS administration of alang-alang root extract with F3 concentration has activity in increasing lymphocyte proliferation, so it can potentially increase the adaptive immune response.

Previous studies conducted *in vivo* showed that alang-alang root extract also showed immunomodulatory effects by increasing the proliferation of cells in lymphocytes[39]. Research on the activity test of the alang-alang root extract *in vivo* also showed differentiation and proliferation, resulting in the enlargement of the lymph[40]. Lymphocytes are the main cells that play a role in the adaptive immune response. This cell is divided into three types: T lymphocytes, B lymphocytes, and NK cells. Animal tests have shown that alang-alang root decoction can increase the proportion of CD4+ T cells in immunodeficient mice, reducing the proportion of CD8+ T cells to balance and restore immune system function. In addition, alang-alang root decoction can also increase the proportion of T helper cells (Th cells) in the spleen of mice and increase IL-2 secretion[33].

4. CONCLUSION

The ethanol extract (*Imperata cylindrical* (L.) P.Beauv.) contains 82 compounds, with the most significant percentage of compounds being cis-13-Octadecenoic (oleic acid), which is 22.73% and 5-Hydroxymethylfultural of 17.38%. The administration of SNEDDS of alang-alang root extract (*Imperata cylindrica* (L.) P.Beauv.) has immunostimulant activity on macrophage phagocytosis activity and lymphocyte cell proliferation in the form of an increase in the value of the largest phagocytosis capacity in formula F3 (250mg) by 84%, the most extensive macrophage phagocytosis index in formula F3 (250mg) by 3.68 and the most extensive lymphocyte stimulation index in formula F3 (250mg) by 3.33 compared to the control cell. The limitation of this study is that it only looks at immunostimulatory activity on macrophage and lymphocyte activation. So that further research can look at several other immunostimulant parameters.

5. ACKNOWLDEGEMENT

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethic Approval

This research was reviewed and approved by the Ethical Committee, under the approval number 039/EC-HC-KEPK FKIK-UMY/IX/2023.

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