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Potential of Fig Leaf (*Ficus carica* L.) as Immunomodulatory Agent *in vitro* and in Silico

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Abstract: Natural remedies are increasingly being used as alternatives to conventional medicine, and their popularity is growing. The fig plant (*Ficus carica* L.), which is known to contain secondary metabolites such as alkaloids, flavonoids, phenolics, terpenoids, steroids, and saponins, has the potential to act as an immunomodulator. The purpose of this study was to determine the content of flavonoid compounds based on Thin Layer Chromatography (TLC), total flavonoid content, total phenolic content, and immunomodulatory activity of ethanol extract and fig leaf fraction *in vitro* and silico (molecular docking). The fig leaf powder was extracted with 70% ethanol and fractionated with n-hexane and ethyl acetate to obtain ethanol extract, ethyl acetate fraction, n-hexane fraction, and water fraction of fig leaves. The extracts and fractions were then identified using the TLC method, and the total flavonoid and total phenolic levels were measured using the colorimetric method. Furthermore, the proliferation of lymphocyte cells and macrophage phagocytic activity were used to gauge the *in vitro* immunomodulatory activity. The results showed that the ethyl acetate fraction sample contained the highest total flavonoid and total phenolic content, namely 3.4630±0.04 mgQE/g sample and 220.1801±0.604 mgGAE/g sample. The immunomodulatory activity test findings revealed that fig leaf extract and fraction might enhance macrophage phagocytic activity in comparison to control cells. In the lymphocyte proliferation test, the value of IS>3 in the ethyl acetate fraction indicates that it has lymphocyte proliferation activity. This study showed that fig leaf extract and fraction could increase the phagocytic activity of macrophage cells. The ethyl acetate fraction of fig leaf could increase lymphocyte cell proliferation *in vitro* and silico.

Keywords: Fig Leaves, Total Flavonoids, Total Phenolics, Immunomodulators, *in vitro* and Silico

Introduction

A series of body mechanisms in protecting against infection by identifying and killing pathogenic substances is called the immune system (Sudiono, 2014). The body's defense system can be activated by providing immunomodulators that can be used to increase a person's immune response (Wulan and Agusni, 2015). One Component that acts as an immunomodulator is flavonoids. The immune system can be boosted by the flavonoid group to fend against attacks from bacteria, viruses, and other organisms.

The use of natural ingredients as an alternative treatment is a trend that is quite popular today and its use is starting to increase. The fig plant is one plant with the ability to modulate the immune system (*Ficus carica* L.). The leaves of the fig plant (*Ficus carica* L.) contain secondary metabolites, namely flavonoids, alkaloids, and tannins (Refli, 2012). The fig plant (*Ficus carica* L.), has been the subject of several investigations and it is known that its leaves contain secondary metabolites such as alkaloids, flavonoids, phenolics, terpenoids, steroids, and saponins (Joseph and Raj, 2011). The flavonoid group is one of the components that are immunomodulators. The immune system can be affected by flavonoid compounds through the growth and activation of T and B lymphocytes, the release of several particular cytokines including interferon-gamma, tumor necrosis factor-alpha, and several other interleukins, activation of the

complement system, and activation of phagocytic cells like macrophages and monocytes (Behl *et al*., 2021).

So as an effort to develop research, a search will be carried out on the potential for increasing the immune system using the leaves of the fig plant (*Ficus carica* L.). This research was conducted to obtain empirical evidence regarding the benefits of fig leaf (*Ficus carica* L.) as an immunomodulator. This research is expected to provide more information about the benefits of fig leaves to the community.

Materials and Methods

Tools and Materials

Tool

Analytical balance (Sartorius®), glassware (Pyrex®), blender/grinder (Phillips®), freeze dryer, rotary evaporator (IKA®), water bath (Memmert®), yellow tip (Brand®), blue tip (Brand®), capillary tube (Pyrex®), UV lamp 254 nm and UV 366 nm, Camag Linomat 5, separating funnel (HERMA®), chamber (GG®), Laminar Air Flow (LabTech®), autoclave (Allamerican®), CO_2 incubator (Heraceus®), inverted microscope (Zeiss®), cuvette (Cuvet dispossible®), hemocytometer (Newbauer®), centrifuge (Sorvall®, America®), petri dish, aluminum foil (Diamond®), oven (Memmert ®), UV-Vis spectrophotometer (SHIMADZU®), micropipette, microplate reader, test tube.

Materials

Samples of fig leaves (*Ficus carica* L.) were taken from the fig tree seed shed in Sewon Bantul, Yogyakarta. The leaves used are dried leaves. The leaves are then ground with a blender until they become Simplicia powder. The experimental animals used in this study were male mice of the BALB/c strain. Materials used for phytochemical analysis included 70% ethanol (General Labora/grade pro analysis), ethyl acetate, n-hexane (General Labora/technical grade), methanol, distilled water, ammonia spray reagent, silica gel 60 GF254, Folin-Ciocalteu reagent, anhydrous aluminum chloride ($AlCl_3$) (Sigma), anhydrous quercetin (Sigma), Na_2CO_3, gallic acid, quercetin, $FeCl_3$, H_2SO_4, HCl.

Materials used for immunomodulator test of fig leaf (*Ficus carica L.*), male BABL/c strain mice 2-3 months old, hepatitis B vaccine, Dimethylsulfoxide (DMSO), latex beads (Sigma) 3 m diameter, Giemsa paint, coverslips round, 24 well microplate (Iwaki), petri dish, 96-well multiwell plate (Nucn, UK), Phosphate-Buffered Saline (PBS), Rosewell Park Memorial Institute (RPMI)-1640 (Sigma) media, Fetal Bovine Serum (FBS), tris ammonium chloride buffer, 0.01 N hydrochloric acid SDS, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide].

Material Preparation and Powder Making

Samples of fresh fig leaves (*Ficus carica* L.) from Yogyakarta were collected. Furthermore, wet sorting was carried out to be separated from dirt or foreign material to reduce the number of impurities carried in the test material. The fig leaves were washed with running water so that no dirt was attached. Then the leaves were drained and the wet weight was weighed. Fig leaves were dried in an oven at a temperature of 50-60°C for 24 h to dry. Then, the dry weight of the simplicia was weighed. Simplicia was blended to get the powder and then weighed.

Ethanol Extract Manufacturing

Simplicia powder of approximately 1 kg was dissolved in 6 L of a 70% ethanol solution. The solution was stirred and stored for 24-48 h. The solution was filtered and obtained a liquid extract and pulp. For optimal results, the powder was macerated once. The macerate was evaporated with a rotatory evaporator and a water bath at a temperature of 60°C. A thick extract was then obtained (Munawaroh *et al*., 2018).

Every 5 g of thick extract of fig leaf (*Ficus carica* L.) was dissolved in 50 mL of 9:1 v/v methanol-water mixture then 50 mL of n-hexane was used to liquid-liquid partition the mixture until the n-hexane phase was clear. The n-hexane phase was separated and evaporated using a rotary evaporator at 50°C. The methanol-water phase was evaporated using a water bath at 60°C until thick and suspended in distilled water. Every 100 mL suspension was partitioned liquid-liquid using 100 mL of ethyl acetate until it reached the clear phase of the ethyl acetate phase. A rotary evaporator operating at 50°C was used to separate and evaporate the ethyl acetate phase. The aqueous phase is filtered to separate the insoluble material. The aqueous phase was evaporated using freeze-drying. The insoluble material was dissolved in 96% ethanol and evaporated using a water bath at 60°C. The fractions obtained from the fractionation are n-hexane, ethyl acetate, and water (Munawaroh *et al*., 2018).

Analysis of Compound Content: TLC-Densitometry

The content of flavonoid secondary metabolites in fig leaves (*Ficus carica* L.) was identified by Thin Layer Chromatography (TLC). The standard compounds used are quercetin and rutin. The stationary phase used is silica gel plate GF254. The fraction was spotted on the stationary phase in Camag Linomat and then eluted with the mobile phase BAW (n-butanol: Acetic acid: Water) in a ratio of 3:1:1. After that, the silica gel plate is dried. Rf values were calculated afterward spots were seen under UV light at 254 and 366 nm.

Furthermore, the test results that have been observed for the content of the compounds using the Thin Layer Chromatography (TLC) method were followed by an analysis of the content of the compounds using a densitometry tool. The study determined and compared each test compound's chromatogram profile and UV profile and compared them to standard compounds of quercetin and routine standards (Munawaroh *et al*., 2018).

Total Flavonoid Level Determination Test

Stock solution preparation was done by weighing as much as 50 mg and dissolving in 100 mL of distilled water to obtain a 500 g/mL stock concentration. The graded series was made with a concentration of 2; 3; 4; 5 and 6 g/mL in 5 mL of distilled water. The determination of the maximum wavelength obtained was 431 nm. The total flavonoid content was calculated by dissolving the extracted sample and fractioning 20 mg in 1 mL to get 20,000 g/mL stock. The stock solution was taken as much as 0.5 mL plus 1.5 mL of methanol, 0.1 mL of $AlCl_3$, and 0.1 mL of Na Acetate and then added with distilled water up to 5 mL. The levels were established for three replications of the measurement in each sample and the absorbance was measured at a wavelength of 431 nm (Munawaroh *et al*., 2018).

Total Phenolic Level Determination Test

The phenolic test was carried out by weighing 100 mg of extract and fraction and then diluting to 10 mL with the solvent to obtain a 10 mg/mL concentration. Pipette 1 mL and dilute with distilled water to 10 mL and get a 1 mg/mL concentration. Pipette 0.2 mL of extract, add 15.8 mL of distilled water and 1 mL of Folin-Ciocalteu reagent, then shaken. Let stand for 8 min, then add 3 mL of 10% Na_2CO_3 to the mixture. The solution was allowed to stand for 2 h at room temperature. The absorption was measured using a UV-Vis spectrophotometer at a maximum absorption wavelength of 726 nm. It was carried out three times to get the phenol content as mg gallic acid equivalent/g fresh sample (Orak, 2006).

Macrophage Phagocytosis Activity Test by in vitro Methods

The *in vitro* study of immunomodulatory has ethical clearance approval from health research ethics committee with number: No.065/EC-KEPK FKIK UMY/XI/2021. Male Balb/c mice that were 2-3 months old were sacrificed using chloroform and the abdominal cavity was cut open. Macrophages were isolated by injecting 10 mL of cold RPMI 1640 medium into the peritoneal cavity while gently shaking for 5 min. The liquid was removed and centrifuged at 1200 rpm. Then, 3 mL of complete RPMI-1640 medium was added to the pellet sediment. The cells were counted and resuspended in a complete RPMI medium to produce a cell suspension with 2.5 × 106 cells/mL density. On 24 well plates that had been given round coverslips, cell suspensions were plated; each well was 200 L, allowed to stand for 30 min, then added with complete RPMI medium 800 L/well and incubated in a 5% CO_2 incubator at 37°C for 24 h (Munawaroh *et al*., 2018).

The media was then taken so that there were only macrophages in the coverslips. The sample material of the concentration series test (fractions 125; 250; 500; 750 g/mL), control cells, added as much as 500 L/well for three replications, was incubated for 4 h. The test material was removed and washed once with an RPMI-1640 medium. Latex suspension with a concentration of 2.5 × 107/mL in complete RPMI medium was added as much as 200 L to each well and then incubated for 60 min. Cells were washed with PBS twice, let to dry at room temperature, and then fixed for 30 sec with methanol. After removing the methanol, the coverslips were allowed to dry before being stained for 20 min with 10% Giemsa in distilled water, thoroughly cleaned with distilled water, and allowed to dry at room temperature. Phagocytic Capacity (PC) and Phagocytic Index (PI) were used to analyze the phagocytic activity of macrophages (Munawaroh *et al*., 2018).

Lymphocyte Proliferation Activity Test by in vitro Methods

Male BALB/c strain 2-3 months mice were sacrificed using chloroform. The upper and lower parts of the abdominal cavity of the mice were cleaned with 70% alcohol, then dissected and the spleen was taken. In a petri dish containing RPMI 1640, the spleen was put. The suspension was centrifuged at 3200 rpm, at 4°C for 4 min. The supernatant was separated and the pellet obtained was suspended in Tris Buffered Ammonium Chloride and allowed to stand at room temperature for 15 min. RPMI was added and centrifuged again for 4 min then the supernatant was discarded. Washing was done two times with RPMI, suspended in complete medium. Cell counts were performed using a hemocytometer and an inverted microscope. Cells were incubated in a 5% CO_2 incubator at 37°C (Winanta, 2017).

Subsequently, the incubated cells were suspended in a complete medium and distributed 100 L each on a 96-well multiwall plate. A total of 10 mL of hepatitis B vaccine was added to each well and incubated for 24 h. The test sample was added to the well and incubated for 48 h. In each well was added 10 L of MTT solution in sterile PBS and incubated again at 37°C for 4 h. Then, to each well, a stopper reagent (10% SDS) was added in 50 mL HCl 0.01 N. The proliferation Stimulation Index (IS) was calculated, and absorbance was measured using a microplate reader at a wavelength of 550 nm (Hertiani and Sasmito, 2013).

Activity Test in Silico

Molecular Operating Environment (MOE) application download. Protein structure retrieval through the Protein Data Bank (PDB) with PDB ID for interferon (5EH1), tumor necrosis factor (2AZ5), and interleukin-2 (1M48). The stages of the docking procedure include ligand preparation, protein preparation, and docking simulation.

Results

Compound Content Analysis by Thin Layer Chromatography Method

Several tests have been carried out to produce a phytochemical profile of each sample utilized in this research and to identify the chemicals that are responsible for the activity. The TLC method was applied to the extracts to provide information on the phytochemical profiles of the 70% ethanol extract, ethyl acetate fraction, water fraction, and n-hexane fraction of fig leaves.

Figure 1 showed that the 70% ethanol extract, the aqueous fraction, the ethyl acetate fraction, and the n-hexane fraction had Rf values in the same range as the quercetin standard, 0.94. However, in 70% ethanol extract, both under UV 254 nm and UV 366 nm, spots appeared at Rf 0.71, similar to the standard Rf of rutin. In the water fraction, spots also appeared with an Rf value of 0.65. The value was almost the same as the standard quercetin, 0.72.

Compound Content Analysis by Thin Layer Chromatography-Densitometry Method

The chromatogram profile of the densitometric TLC results can be seen in Fig. 2. The chromatogram pattern of standard quercetin, ethyl acetate fraction, and n-hexane fraction showed almost similar patterns. Meanwhile, in the other chromatogram pattern, the 70% ethanol extract sample had a pattern nearly the same as the water fraction sample, which shows the results of 2 peaks. As for the routine chromatogram pattern, it offered a different pattern itself.

The standard used in this TLC-densitometry test was quercetin and rutin. The following data were obtained by comparing the peaks of the chromatogram profile between the standards and all sample fractions. At peak 2, water fraction, ethyl acetate fraction, and n-hexane fraction, the ethanol extract had peaks similar to the standard quercetin Rf 0.82-0.99. These results indicate that the sample content of water fraction, ethyl acetate fraction, n-hexane fraction, and 70% ethanol extract is most likely to contain flavonol compounds (quercetin). The samples of the ethanol extract and the water fraction at the 1st peak had a peak similar to the standard routine Rf 0.57-0.81. This finding shows that the 70% ethanol extract sample and the water fraction also most likely contain flavonoid glycosides (routine) compounds. The UV profile of each fig leaf test compound can be seen in Fig. 3.

The UV profile of these compounds can be used to determine whether the compounds are a type of flavonoid (Fig. 3). Identification was carried out by comparing the peak bands 1 and 2 of each compound wave pattern tested with the peak band of flavonoid compounds. UV profile data from 70% ethanol extract and aqueous fraction, ethyl acetate fraction and n-hexane fraction from leaves showed the maximum wavelength of each band. In Band I, the maximum wavelength range is 310-350. Meanwhile, band II which is included in the maximum wave is 250-280. These data indicate that in the second wave of 70% ethanol extract and water fraction, the ethyl acetate and n-hexane fractions most likely contain flavonoid compounds of the flavone type.

Measurement of Total Flavonoid Levels Analysis

Analysis of total flavonoid levels measurement (Table 1) showed that the ethyl acetate fraction had the highest flavonoid content of 3.4630±0.04 compared to the total flavonoid content of the n-hexane and water fractions were 1.893±0.21 and 2.1697±0.002, respectively.

Analysis of Measurement of Total Phenolic Levels

The measurement of total flavonoids result (Table 2) showed that the ethyl acetate fraction had the highest total phenolic content of 220.1801±0.604 compared to the total phenolic content of the n-hexane and water fractions, which were 34.981±1.76 and 27.591±0.06, respectively.

In Silico Test

The in silico test was conducted with molecular docking on the ethanol extract of fig leaves containing quercetin and luteolin compounds and using comparisons derived from the plant *Echinacea purpurea*, namely *chicoric acid* and arabinogalactan, against the target proteins of IFN-γ, TNF-α, and IL-2.

The results (Table 3) showed that the comparison compounds, namely chicoric acid and arabinogalactan, had a higher docking score than quercetin and luteolin as the test compounds. The compounds of chicoric acid and arabinogalactan had a lower bond affinity with better bond energy than quercetin and luteolin.

Immunomodulatory Activity: Macrophage Phagocytosis Test

The phagocytic activity of the macrophages test result is described in Fig. 4(a-b). The result indicated the value of the phagocytic capacity profile and the macrophage phagocytosis index.

The results shown in Fig. 4 (a-b) are increased phagocytic capacity activity and phagocytic index of several

levels compared to control cells, which means that fig leaf extract and fraction can increase the phagocytic activity of macrophage cells. The ethanol extract of fig leaves with the highest PI and PC was found at 500 g/mL (PI = 5.06±0.40; PC = 84%±0.02). Judging from the IF value, it can be concluded that the ethanol extract from fig leaves can increase endurance. At a concentration of 750 g/mL, the n-hexane fraction showed the maximum phagocytic capacity (PC = 86.67%±1.88). The maximum phagocytosis index was reported in the water fraction at a concentration of 750 g/mL (PI = 4.03±0.45). There have not been many studies of fig leaf extracts and fractions related to isolating active compounds with immunomodulatory effects. The fig plant (*Ficus carica* L.), has been the subject of numerous investigations and it is known that its leaves contain secondary metabolites such as alkaloids, flavonoids, phenolics, steroids, saponins, and terpenoids (Joseph and Raj, 2011). Meanwhile, another study conducted by Mali and Hatapakki (2008) showed that Centella Asiatica Linn. with the content of active substances triterpenoids and saponins *in vitro* have immunomodulatory activity (Mali and Hatapakki, 2008).

Table 1: Test results of ethanol extract and tin leaf fraction total flavonoid level

Sample	mgQE/g sample
Ethanol extract	1.286±0.005
Ethyl acetate fraction	3.463±0.040
N-hexane fraction	1.893±0.210
Water fraction	2.169±0.002

Table 2: Results of total phenolic concentration of ethanol extract and tin leaf fraction

Sample	mgGAE/g sample
Ethanol extract	47,538±0.450
Ethyl acetate fraction	220,180±0.604
N-hexane fraction	34,981±1.760
Water fraction	27,591±0.060

Table 3: Molecular docking test results between ligands and receptor proteins

	Docking score (kcal/mol)		
Test ligan	IFN- γ	TNF-α	IL-2
Quercetin	-4, 1708	-6, 7737	-5, 7640
Luteolin	-4, 3544	-6, 4892	-5, 2284
Chicoric acid	-5, 1539	-8, 3043	-7, 2189
Arabinogalactan	-4, 2899	-9, 0707	-6, 0790

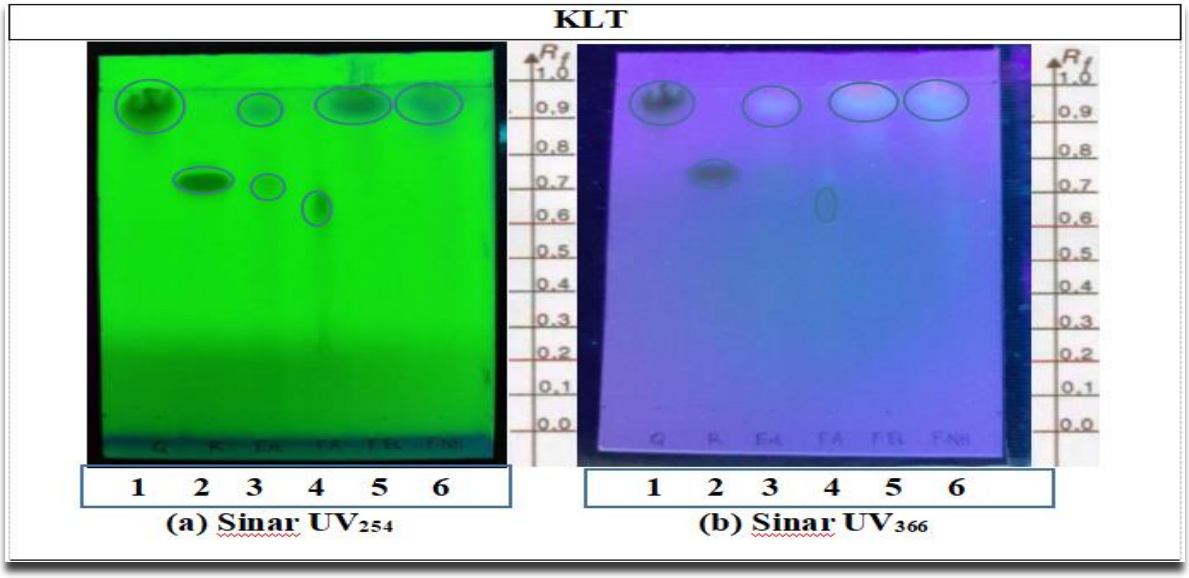


Fig. 1: TLC profile (1) Quercertin standard, (2) Rutin standard, (3) Ethanol extract 70% fig leaf, (4) Water fraction, (5) Ethyl acetate fraction, (6) N-hexane fraction, (a) Under UV 254, (b) Under UV 366. Remarks: TLC system, stationary phase: GF254,B: A: W (n-butanol: Acetic acid: Water) as mobile phase

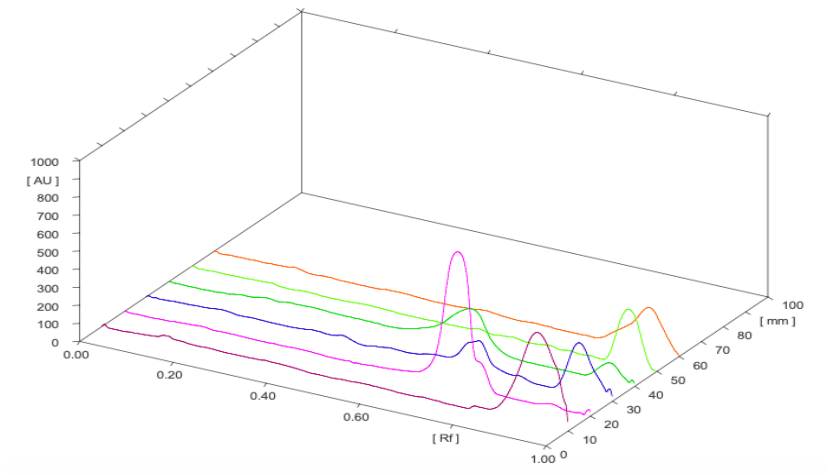


Fig. 2: Chromatogram profile of samples of quercetin (pink), rutin (purple), ethanol extract (blue), water fraction (green), ethyl acetate fraction (yellow) and n-hexane fraction (orange color) fig leaves

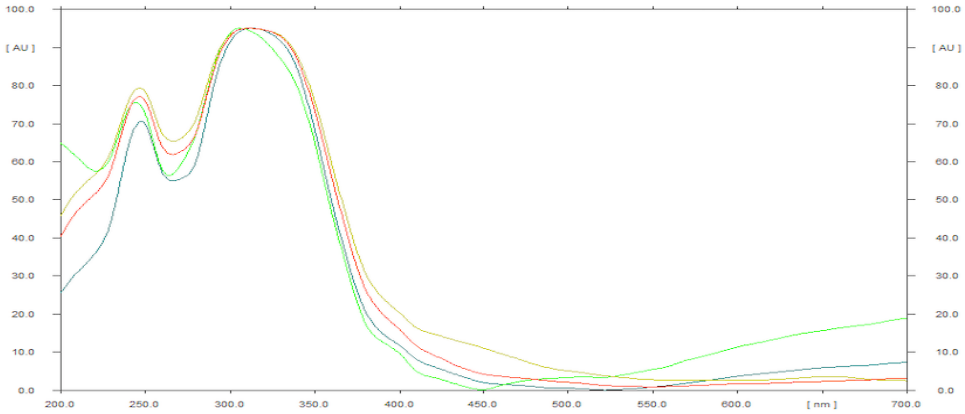
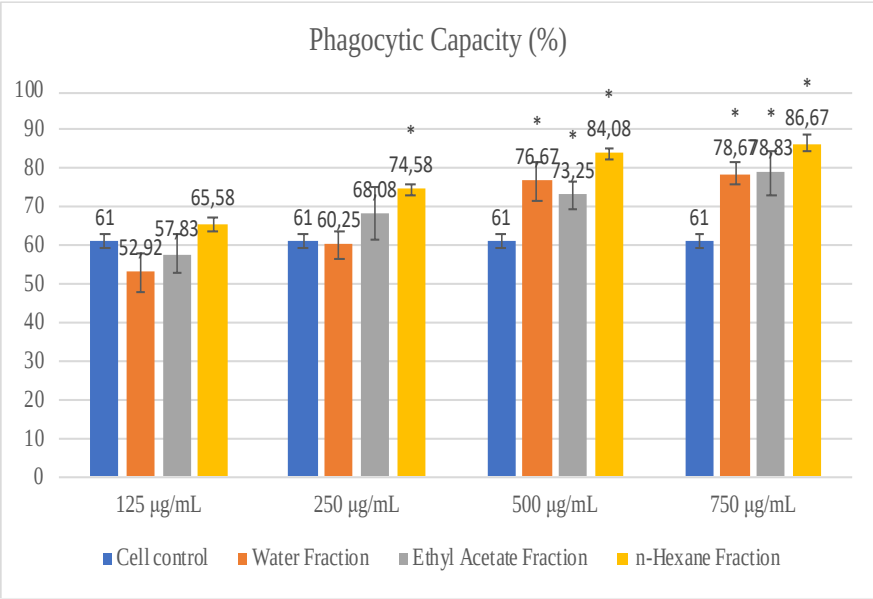
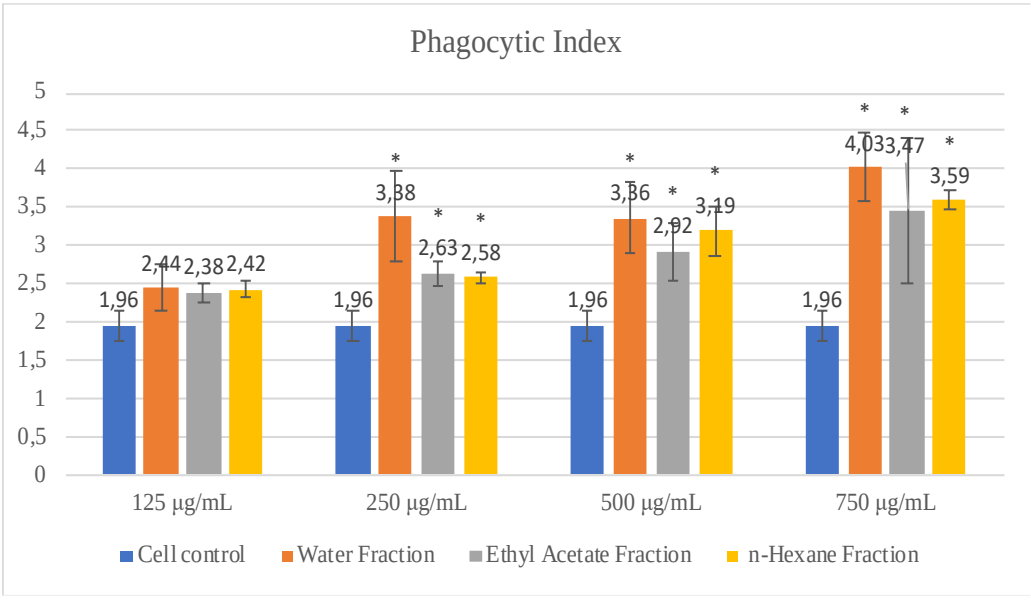


Fig. 3: UV profile of maximum wavelength on each test compound with Rf 0.70-0.90



(a)



(b)

Fig. 4: Profile of macrophage phagocytic capacity (a-b) Macrophage phagocytosis index due to fig leaf ethanol extract, water fraction, ethyl acetate fraction, and n-hexane fraction of fig leaf extract. *Significant difference to control ($p<0.05$)

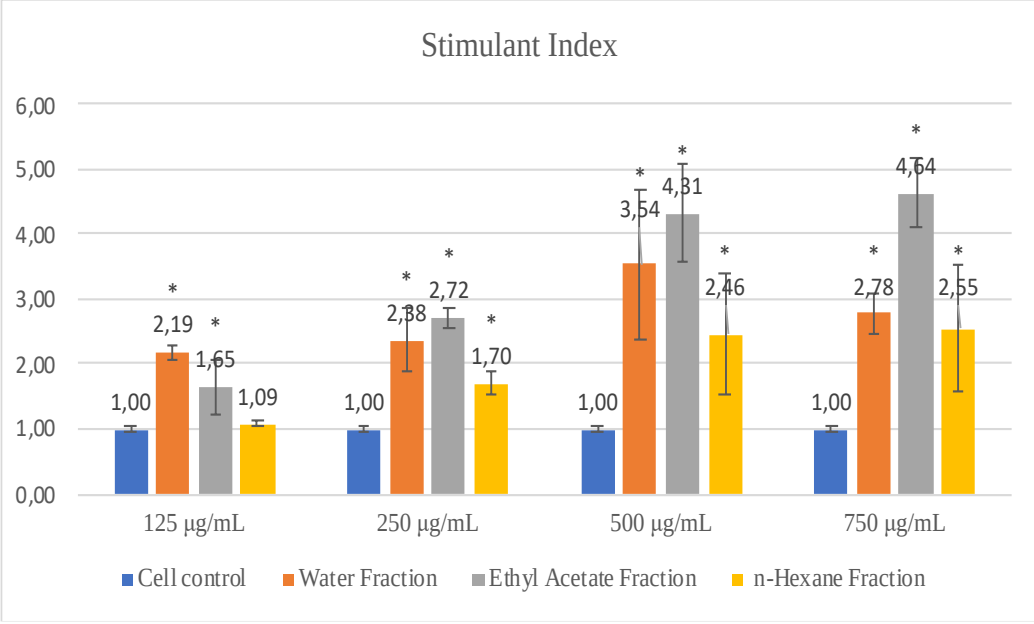


Fig. 5: Profile of lymphocyte proliferation stimulant index due to the administration of ethanol extract, water fraction, ethyl acetate fraction, and n-hexane fraction of fig leaves. *Significant difference to control ($p<0.05$)

Immunomodulatory Activity: Lymphocyte Proliferation Test

Lymphocyte proliferation is a process that describes the response of lymphocytes to antigenic stimuli. In contrast, lymphocytes serve as both humoral and cellular immunity mediators, particularly recognizing and reacting to foreign antigens. The results of experiments evaluating the effect of applying all types of samples on lymphocyte proliferation indicated the proliferation stimulation index.

The results of the lymphocyte proliferation test shown by the stimulation index profile Fig. 5 have provided an overview of the proliferation activity of lymphocytes based on variations in concentration. Based on Fig. 5, the proliferation activity of lymphocyte cells based on

variations in concentration shows that the administration of the ethyl acetate fraction, water fraction, and n-hexane fraction resulted in a proliferation index of more than 2. The lymphocyte proliferation index of a drug compound, in general, is known as SI. SI value between 2 and 3 is considered a weak positive and considered positive when the SI value is greater than 3 (SI>3), mainly if a positive result is obtained in more than one concentration (Pichler and Tilch, 2004). These results indicate that the administration of fig leaf extract is thought not to affect the increase of mice's lymphocyte proliferative activity. So this study shows that this fraction has activity in increasing lymphocyte cell proliferation and has the potential to increase the adaptive immune response. Administration of the ethyl acetate fraction with a 750 µg/mL concentration positively increased lymphocyte proliferative activity with an SI value of 4.64.

Discussion

It has been reported in previous studies that flavonoids boost the immune system's biochemical and pharmacological functions as well as those of cells involved in inflammation, such as T cells, B cells, macrophages, neutrophils, mast cells, and basophils. Flavonoids can influence the inflammatory process. These enzymes contribute to signal transduction, T-cell proliferation, activation of B cells, and cytokine production processes that stimulate monocytes (García-Lafuente *et al*., 2009).

There have not been many studies of ethanol extract, ethyl acetate fraction, water fraction, and n-hexane fraction of fig leaves related to isolating active compounds with immunomodulatory effects. Munawaroh *et al*. (2018) stated that the ethyl acetate fraction of the ethanolic extract of faloak bark containing flavonoid compounds had macrophage phagocytic activity (Munawaroh *et al*., 2018). Another study, (Handayani, 2018) tested the effect of ethanol extract of suji leaves containing flavonoid compounds to increase the phagocytosis index of macrophages. Administration of raw lompong extract (*Colocasia esculenta* L. Schoot) to Balb/c mice can increase the average index of phagocytosis (Sulistiani and Rahayuningsih, 2015). Winanta and Hertiani (2019) have also observed the activation of no production in macrophages after the dekokta treatment of Faloak bark.

This study analyzed the relationship between the content of flavonoids and phenolic compounds with immunomodulatory activity to stimulate specific and non-specific immune responses. The non-specific immune response is indicated by the phagocytic activity of macrophages. The specific immune response is characterized by the activity of lymphocyte proliferation. However, the activity is not only determined by the group of flavonoid and phenolic compounds because other compounds can also contribute to this activity. Macrophage cells have an essential role in the immune response, namely phagocytosis or eating foreign particles such as microorganisms, macromolecules including antigens, and even damaged or dead cells or tissues themselves (Abbas *et al*., 2019; Shen and Louie, 1999).

The flavonoids contained in fig leaves can stimulate the production of IL-2. IL-2 plays a significant role in activating T lymphocyte cells to proliferate. The binding of IL-2 regulates the proliferation of T lymphocytes stimulated by antigens to its receptor. IL-2, besides activating T lymphocyte cells, also plays a role in stimulating the proliferation and differentiation of B lymphocytes and Natural Killer (NK) cells (Baratawidjaja and Rengganis, 2012). This study shows that fig leaf extract and fraction increase phagocytic capacity activity and phagocytic index for several levels compared to control cells. This finding means that fig leaf extract and fraction could increase the phagocytic activity of macrophage cells. This study indicates that the extract and fraction of fig leaves can potentially stimulate non-specific immune responses.

Several studies were conducted to examine the effect of plant extracts on lymphocyte cell proliferation. Kanjwani *et al*. (2008) investigated an extract of Piper betel L. IFN-γ, a cytokine essential for the development of cytotoxic T cell precursors in T lymphocyte activation, differentiation, and maturation. The results of this study indicate that suppression of lymphocyte cell proliferation (immunosuppressants), both in cellular and humoral immune responses from piper betel extract, may mediate the immunosuppressant activity (Kanjwani *et al*., 2008). The water, ethyl acetate, and n-hexane fraction resulted in a proliferation index of more than 3. This study showed that these fractions have activity in increasing lymphocyte cell proliferation and have the potential to increase adaptive immune responses.

Conclusion

In general, based on the results of phytochemical tests, the ethyl acetate fraction has the highest total flavonoid and phenolic content. The extract, n-hexane fraction, ethyl acetate fraction, and water fraction of fig leaves showed an effect in increasing the phagocytic activity of macrophages. As for lymphocyte proliferation activity, only the fig leaf fraction showed efficacy in improving the proliferation of lymphocyte cells. The results of this study have the potential as scientific evidence of the effectiveness of fig leaves and can be used as precursors and benchmarks for the development of new drugs as immunomodulatory agents. This research will enable researchers to investigate crucial areas that many others are unable to investigate regarding the potential benefits of fig leaves in improving innate immune responses and adaptive

immune responses. Thus a new theory of the immunomodulatory activity of fig leaves can be obtained.

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Author's Contributions

Anita Dewi: Principal researcher.

Fitri Rahmayanti: Conducting TLC test, total flavonoids, and total phenolics.

Haidar Bagir: Conducting macrophage phagocytosis test.

Asni Fathul Jannah: Conducting lymphocyte proliferation test and molecular docking test.

Aji Winanta: Principal researcher and supervisor.

Ethics

This manuscript has not been partially or fully published elsewhere and no other journal is considering it. The submission of the manuscript to this publication has the approval of all authors. The authors declare that there are no ethical concerns or potential conflicts of interest following the publication of this study.

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