



Immunosuppressive Effect of Methanol Extract and Ethyl Acetate Fraction of Breadfruit (*Artocarpus altilis* (Park.) Fosberg) Leaves

(Efek immunosupresif ekstrak metanol dan fraksi etil asetat daun sukun (*Artocarpus altilis* (Park.) Fosberg))

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Article Info:

Received: 22 July 2022

in revised form: 23 July 2022

Accepted: 4 October 2022

Available Online: 18 October 2022

Keywords:

Artocarpus altilis

Methanol extract

Ethyl acetate fraction

Immunosuppressant

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ABSTRACT

Background: *Artocarpus sp.* contained a variety of flavonoids, some of which have been shown to reduce inflammation. In vitro anti-inflammatory activity of breadfruit (*Artocarpus altilis* (Park.) Fosberg) has been demonstrated. Chronic inflammation can occur when immune defence mechanisms are activated by inflammatory conditions. **Objectives:** This study examined the immunosuppressive effects of breadfruit leaf methanol extract and ethyl acetate fraction. **Material and Methods:** The bioactive compounds in breadfruit leaves were extracted in methanol via maceration and fractionation in ethyl acetate via liquid-liquid extraction. Phytochemical screening was performed by chemical reagents. The in vivo immunosuppressive effect was evaluated on male Swiss albino mice by humoral antibody titer, delayed-type hypersensitivity, and phagocytic index assays. **Results:** According to phytochemical screening, flavonoids and steroids were found in the methanol extract and its ethyl acetate fraction. The breadfruit leaf methanol extract and fraction showed an immunosuppressive effect through phagocytic index < 1, and the thymus gland index was significantly decreased ($p < 0.05$) compared to control. Both were able to inhibit the production of antibodies and DTH reactions, two types of immune responses. **Conclusions:** The methanol extract of breadfruit leaves and the ethyl acetate fraction showed immunosuppressive properties.



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How to cite (APA 6th Style):

Palupi, D. H. S., Soemardji, A. A., Iwo, M. I. (2022). Immunosuppressive Effect of Methanol Extract and Ethyl Acetate Fraction of Breadfruit (*Artocarpus altilis* (Park.) Fosberg) Leaves. *Jurnal Farmasi Galenika: Galenika Journal of Pharmacy (e-Journal)*, 8(2), 124-131. doi: 10.22487/j24428744.2022.v8.i2.15946

ABSTRAK

Latar Belakang: *Artocarpus* sp. mengandung berbagai flavonoid, beberapa di antaranya telah terbukti mengurangi peradangan. Aktivitas anti-inflamasi sukun (*Artocarpus altilis* (Park.) Fosberg) secara *in vitro* telah dibuktikan. Peradangan kronis dapat terjadi ketika mekanisme pertahanan kekebalan diaktifkan oleh kondisi peradangan. Tujuan: Penelitian ini mengkaji efek immunosupresif ekstrak metanol daun sukun dan fraksi etil asetat. Bahan dan Metode: Senyawa bioaktif dalam daun sukun diekstraksi dalam metanol melalui maserasi dan fraksinasi dalam etil asetat melalui ekstraksi cair-cair. Skrining fitokimia dilakukan dengan pereaksi kimia. Efek immunosupresif *in vivo* dievaluasi pada tikus albino Swiss jantan dengan titer antibodi humoral, hipersensitivitas tipe lambat, dan uji indeks fagositik. Hasil: Skrining fitokimia menunjukkan adanya flavonoid dan steroid dalam ekstrak metanol dan fraksi etil asetat daun sukun. Ekstrak dan fraksi daun sukun menunjukkan efek immunosupresif melalui indeks fagositosis <1, dan indeks kelenjar timus menurun secara signifikan ($p < 0,05$) dibandingkan dengan kontrol. Baik ekstrak metanol daun sukun maupun fraksi etil asetat mampu menghambat produksi antibodi dan reaksi DTH. Kesimpulan: Ekstrak metanol daun sukun dan fraksi etil asetat menunjukkan sifat immunosupresif.

Kata kunci: *Artocarpus altilis*, ekstrak metanol, fraksi etil asetat, immunosupresan

INTRODUCTION

Inflammation is the body's normal reaction to microbial infections, tissue injury, and other dangerous situations. That's a normal immune system response to ensure the organism's survival. Uncontrolled or unresolved inflammation can lead to tissue damage, metabolic syndromes, autoimmunity pathologies, and organ function loss (Sugimoto *et al.*, 2016). Stabilizing inflammation early and minimizing tissue damage are requirements for an excellent medication for treating immune-mediated inflammatory illnesses (Serhan, 2017). Immunosuppressants work by inhibiting the immune system, which helps to reduce or eliminate inflammation and damage to cells. These medications lessen the effects of the symptoms. Even autoimmune diseases can be put into remission by these treatments (Duan *et al.*, 2019).

Natural products have traditionally been used to find bioactive pharmaceuticals, including immunomodulators. Flavonoids are polyphenolic compounds with antioxidative, anti-inflammatory, memory-enhancing, cardiovascular and gastroprotective effects (Huang *et al.*, 2010). Breadfruit [*Artocarpus altilis* (Park.) Fosberg], well-known in Indonesia as Sukun, is a tropical plant in the family Moraceae. *A. altilis*, besides being a food source, is also frequently utilized in traditional medicine. According to research conducted on this plant's bioactive compound, secondary metabolites can be found in breadfruit in various forms, including flavonoids, stilbenes, steroids, and lectins. Breadfruit extracts and isolated chemicals have anti-inflammatory, immunomodulatory, antioxidant, anticholinergic, and cytotoxic properties (Sikarwar *et al.*, 2014). However, breadfruit leaves have never been studied for their effects as an immunosuppressant. Therefore, this study aimed to examine the immunosuppressive properties of the methanol extract and the ethyl acetate fraction of breadfruit leaves.

MATERIAL AND METHODS

Materials

The leaves of *Artocarpus altilis* were taken from the Dago Region in the Bandung District of West Java, Indonesia. Only the yellow-coloured leaf still attached to the plant at the time of sampling was used in this study. The plant was identified at Herbarium Bandungense, School of Life Science and Technology, Bandung Institute of Technology. n-hexane, ethyl acetate, ammonia, toluene, chloroform, hydrochloric acid, dragendorff reagent, mayer reagent, stiasny reagent, saline phosphate buffer, and sheep red blood cells (srbc) were purchased from PT. Biofarma.

Methods

Animals

Female swiss mice between 1-2 months old were chosen. The animals were kept in standard conditions (12 hours of light and 12 hours of dark, a temperature of $25\pm 2^{\circ}\text{C}$, and relative humidity of $50\pm 15\%$) at the animal laboratory in the School of Pharmacy Institut Teknologi Bandung. All animal experiments were done with the approval of the Institut Teknologi Bandung's ethics committee for experimental use with the letter number 01-02/kephp-itb/11-2017.

Extraction

About 1 kg of breadfruit leaf powder was extracted by maceration method using methanol as solvent for 3 x 24 h. Then, the extract was filtered. The filtrate was dried with a rotary vacuum evaporator to make a thick extract. The thick methanol extract was successively extracted by n-hexane and ethyl acetate as solvents by liquid-liquid extractions. The ethyl acetate fraction was taken and evaporated to obtain a thick fraction.

Phytochemical screening

The color reactions were used in the qualitative phytochemical screening of the extracts to find the main chemical groups (alkaloids, tannins, saponins, terpenoids, flavonoids, and phenols) in the extracts.

Treatment Protocol

Mice were randomly separated into five groups of five animals in each group. In group I, animals as negative control were given 1 mL/20g 0.3% w/v tragacanth, and group 2 received 0.045 g/kg methylprednisolone as a standard drug. Three test groups were treated with breadfruit leaf methanol extract at a dosage of 0.63 and 0.1575 g/kg and ethyl acetate fraction 0.1575 g/kg. The negative control, extracts, and standard drug were given orally.

Phagocytic index assay

All of its groups received the treatment once daily, orally for seven consecutive days. On day 8, the mice were intravenously given 0.1 ml of carbon suspension through the tail vein. Blood samples were collected from the retro-orbital plexus before and on 4, 8, 12, 16 and 20 min after immunization. Twenty microliters of blood were mixed with 2 ml of 0.1% acetic acid for erythrocyte lysis, and the absorbance was measured at 675 nm. The obtained data were then processed to determine the rate of carbon particle elimination by constructing a linear regression curve between 100-%T for time. The slope of the regression line indicates the rate of carbon particle elimination.

The mice were euthanized and their liver, spleen, and thymus glands were isolated to determine their relative weight. Organ indexes were calculated with the following formula:

$$\text{Organ index (\%)} = \frac{\text{organ weight(g)}}{\text{body weight (g)}} \times 100$$

Delayed type hypersensitivity (DTH) assay

The delayed-type hypersensitivity assay was utilized to evaluate the effects of the breadfruit leaves extract and ethyl acetate fraction on the function of the cell-mediated immune system. The extract and ethyl acetate fraction of the breadfruit leaves were given five days before the delayed type hypersensitivity assay was performed.. All groups were immunized intraperitoneally with 0.1 mL/10 g SRBC 1% (day 0). On the sixth day, the thickness of the left foot paw was measured and immunized intradermally with 0.05 mL of 1% SRBC. Foot thickness was measured after 24 and 48 h of the challenge. The difference between the thickness of the left foot before and after the challenge was taken as a measure of DTH, expressed in mm.

Humoral antibody (HA) titer assay

The animal groups were immunized with 0.1 ml/10 g SRBC 1% intravenously (day 0). Mice were administered the test substance orally daily for 12 days. Blood was retrieved from the mice through the tail vein on the fifth day after immunization and centrifuged at 4000 rpm for 10 min (4 °C) to obtain serum. The serum samples were serially diluted twice with 25 µl of 0.9% saline in a microtiter plate, and 25 µl of 1% SRBC in normal saline was added to each serially diluted well and mixed. After 2 hours of incubation at 37°C, the antibody titer was determined by taking the highest serum dilution showing haemagglutination.

Data analysis

All values were expressed as mean ± standard error of mean (SEM). Analysis of variance (ANOVA) was used to see how the groups were different from each other. The data were analysed using the statistical analysis system of SPSS.

RESULTS AND DISCUSSION

Secondary metabolites are plant chemical compounds that mostly responsible for pharmacological activity. Phytochemical screening revealed the presence of chemical compounds in the extract, particularly secondary metabolites. The screening results (Table 1) revealed that extract of *Artocarpus altilis* leaves (EAA) and ethyl acetate fraction of *Artocarpus altilis* (FEAA) contained flavonoids and steroid/triterpenoids. Phytochemical screening by Riasari, et al., (2015) also showed the presence of flavonoid and steroid compounds, although it is known that the percentages of both are different at each stage of leaf development. In the present study, yellow leaves were used, considering they had a higher percentage of bioactive compounds.

The effect of the EAA dan FEAA on phagocytic activity by carbon clearance test was shown in table 2. Removal of carbon particles from the bloodstream serves as an indicator of phagocytic activity in reticuloendothelial cells. The phagocytic index (k) of all treatment groups of test materials of low value (< 1) indicated suppression of *in vivo* phagocytic activity.

Table 1. Class of chemical compounds in methanol extract and ethyl acetate fraction of breadfruit leaves

Test of	Result	
	Methanol Extract	Ethyl Acetate Fraction
Alkaloid	-	-
Flavonoid	+	+
Saponin	-	-
Quinone	-	-
Tannin	-	-
Steroid/Triterpenoid	+	+

Table 2. Phagocytic index in mice after administrated of methanol extract and ethyl acetate fraction of breadfruit leaves

Group	Rate of carbon elimination (K)	Phagocytic index (k)	Immunomodulation effect category (Wagner,1991)
Negative Control	-0.3710	1.00	-
Methylprednisolone 0.045 g/kg	-0.1224	0.33	Immunosuppressive effect
EAA 06300 g/kg	-0.2950	0.80	Immunosuppressive effect
EAA 0,1575 g/kg	-0.2738	0.74	Immunosuppressive effect
FEAA 0,1575 g/kg	-0.3774	1.02	Immunosuppressive effect

The organ index value is another parameter used to assess the effect of extracts on non-specific immune responses. The decreasing weight of the liver, spleen and thymus gland indicated a decrease in the immune response. The data on organ index (Table 3) showed that EAA at 0.6300 g/kg, FEAA at 0.1575 g/kg, and methylprednisolone 0.045 g/kg significantly differed in thymus gland index compared to that in the control group. It means that EAA at 0.6300 g/kg and FEAA at 0.1575 g/kg suppress the activity of the thymus gland activity and the production of T cell maturation. Suppression of the immune response has been demonstrated to decrease the organ index.

Table 3. Organ index in mice after administrated of methanol extract and ethyl acetate fraction of breadfruit leaves

Groups	Organ index (%)		
	Liver	Spleen	Thymus gland
Negative Control	4.02 ± 0.32	0.36 ± 0.09	0.49 ± 0.12
Methylprednisolone 0.045 g/kg	4.05 ± 0.23	0.30 ± 0.10	0.38 ± 0.07*
EAA 0,63 g/kg	4.42 ± 0.96	0.378 ± 0.10	0.35 ± 0.06*
EAA 0,1575 g/kg	3.69 ± 0.34	0.44 ± 0.10	0.39 ± 0.06
FEAA 0,1575 g/kg	3.97 ± 0.38	0.25 ± 0.06	0.30 ± 0.04*

Values are mean±SEM, Each value represents the mean of 5 mice;
Statistically significant difference: * p < 0.05 compare to control.

The DTH response and antibody titer were used to determine immunosuppressive activity against a specific immune response. In table 4, 24 hours after injection of SRBC as antigen, the thickness of the foot of EAA and FEAA groups was significantly smaller (p<0.05) compared to that of the control. The percentage change in the thickness of the foot after 48 hours, in the foot of FEAA group is smaller (p<0.05) compared than control.

Table 4. Percentage changes of paw volume footpad thickness of mice after administrated of methanol extract and ethyl acetate fraction of breadfruit leaves

Groups	% thickness changes volume	
	24 hours	48 hours
Negative Control	100.00 ± 0.00	55.55 ± 9.62
Methylprednisolone 0.045 g/kg	50.00 ± 0.00*	50.00 ± 0.00
EAA 0,6300 g/kg	61.11 ± 20.41*	50.00 ± 16.67
EAA 0,1575 g/kg	50.00 ± 16.67*	38.89 ± 9.62
FEAA 0,1575 g/kg	50.00 ± 16.67*	25.00 ± 8.33*

Values are mean±SEM; Each value represents the mean of 5 mice;
Statistically significant difference: * p < 0.05 compare to control.

B lymphocytes and antibodies secreted by plasma cells are vital elements in the humoral immune response (Owen *et al.*, 2013). The antibody synthesis process involves the role of T and B lymphocytes, Therefore, an increase in antibody titer also reflects an increase in the humoral response (Raj & Gothandam, 2015). Antibody titers were determined to evaluate the effect of breadfruit plant extracts on humoral immune responses. Administration of EAA at doses of 0.1575 and 0.6300 g/kg and FEAA doses of 0.1575 g/kg showed a decrease in hemagglutination antibody titers compared to the control group. This result suggests that EAA and FEAA also affect the humoral immune response by suppressing the activity of memory B cells.

Table 5. Total antibody titre in mice after administrated of methanol extract and ethyl acetate fraction of breadfruit leaves

Groups	Hemagglutinin Antibody titre	
	Primary	Secondary
Negative Control	1 : 256	1 : 128
Methylprednisolone 0.045 g/kg	1 : 256	1 : 32
EAA 0,6300 g/kg	1 : 192	1 : 24
EAA 0,1575 g/kg	1 : 256	1 : 16
FEAA 0,1575 g/kg	1 : 256	1 : 16

The results of the phytochemical screening of EAA and FEAA showed the presence of flavonoids and triterpenoids. According to Edewor (2016), triterpenoids and flavonoids are compounds in plants that have the potential to suppress the immune response. Flavonoids and triterpenoids work by inhibiting various transcription factors, modulating the differentiation, proliferation, and activation of T and B cells, increasing the formation of regulatory T cells, suppressing the expression of cytokines and pro-inflammatory enzymes (IL-1, IL-2, IL-6, IL-10, TNF-, 5-lipoxygenase, and human elastase), as well as inhibiting C3 convertase activity in the classical compartment pathway (Brindha *et al.*, 2016).

CONCLUSION

Breadfruit (*Artocarpus altilis* (Park.) Fosberg), is a plant medicine with many different uses in medicine. In this study, it was shown that treatment with breadfruit leaf extract and ethyl acetate fraction significantly decreased inflammation ($p < 0.05$). Both extract and fraction doses were immunosuppressive with a phagocytic index < 1 and a significantly smaller thymus gland index ($p < 0.05$) than the control. Breadfruit leaf extract and its ethyl acetate fraction were able to inhibit antibody production as a humoral response and inhibit DTH reactions as a cellular immune response.

CONFLICT OF INTEREST

It has been confirmed by the authors that there are no potential conflicts of interest.

REFERENCES

- Brindha, P., Venkatalakshmi, P., & Vadivel, V. (2016). Role of phytochemicals as immunomodulatory agents: A review. *International Journal of Green Pharmacy*, 10(1), 1–2.
- Duan, L., Rao, X., & Sigdel, K. R. (2019). Regulation of Inflammation in Autoimmune Disease. *Journal of Immunology Research*, 2019, 1–2. <https://doi.org/10.1155/2019/7403796>
- Edewor, T. I. (2016). A Review: Immunological Potential Of Bioactive Flavonoids And Flavonoid Containing Fractions Isolated From Medicinal Plants. *Cibtech Journal of Bio-Protocols*, 5(2), 7–14.
- Huang, R.-Y., Yu, Y.-L., Cheng, W.-C., OuYang, C.-N., Fu, E., & Chu, C.-L. (2010). Immunosuppressive Effect of Quercetin on Dendritic Cell Activation and Function. *The Journal of Immunology*, 184(12), 6815–6821. <https://doi.org/10.4049/jimmunol.0903991>
- Owen, J. A., Punt, J., Stranford, S. A., & P., J. P. (2013). *Kuby Immunology* (Seventh Ed, p. 534). W. H. Freeman and Company.
- Raj, S., & Gothandam, K. M. (2015). Immunomodulatory activity of methanolic extract of *Amorphophallus commutatus* var. *Wayanadensis* under normal and cyclophosphamide induced immunosuppressive conditions in mice models. *Food and Chemical Toxicology*, 81, 151–159. <https://doi.org/10.1016/j.fct.2015.04.026>
- Riasari, H., Ruslan, K., & Sukrasno. (2015). Metabolite Profile Of Various Development Bread Fruit Leaves (*Artocarpus Altilis*. Parkinson. Fosberg) And The Identification Of Their Major Componens. *International Journal of Pharmaceutical Sciences and Research*, 6(5), 2170–2177.
- Serhan, C. N. (2017). Treating inflammation and infection in the 21st century: New hints from decoding resolution mediators and mechanisms. *The FASEB Journal*, 31(4), 1273–1288. <https://doi.org/10.1096/fj.201601222R>
- Sikarwar, M. S., Hui, B. J., Subramaniam, K., Valeisamy, B. D., Yean, L. K., & Balaji, K. (2014). A review on *Artocarpus altilis* (Parkinson) Fosberg (breadfruit). *Journal of Applied Pharmaceutical Science*, 4(8), 91–97. <https://doi.org/10.7324/JAPS.2014.40818>
- Sugimoto, M. A., Sousa, L. P., Pinho, V., Perretti, M., & Teixeira, M. M. (2016). Resolution of inflammation: What controls its onset? *Frontiers in Immunology*, 7(APR). <https://doi.org/10.3389/fimmu.2016.00160>