Rini Prastiwi-Arginase Inhibitory, Antioxidant Activity and Pharmacognosy Study of Sterculia macrophylla Vent. Leaves

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Original Article

Arginase Inhibitory, Antioxidant Activity and Pharmacognosy Study of *Sterculia macrophylla* Vent. Leaves

Rini Prastiwi[1,2], Berna Elya[1,*], Rani Sauriasari[3], Muhammad Hanafi[4,5], Yesi Desmiaty[5]

ABSTRACT

Objective: The purpose of this study was to investigate the arginase inhibitory activity, antioxidant activity, and also pharmacognostical study of *Sterculia macrophylla* leaves. The main component of genus *Sterculia* was flavonoid that was well known to demonstrate arginase inhibitory activity. **Methods:** Sample was extracted gradually using n-hexane, ethyl acetate, and methanol solvents, subsequently. The n-hexane, ethyl acetate, and methanol extract were determined for their arginase inhibitory activity. The most active extract was methanol extract. This extract was determined for its antioxidant activity, arginase inhibitory activity, identification of chemical compound, chromatogram profile and determined the content of total flavonoid. The leaves and powder of *Sterculia macrophylla* were identified with microscopic and macroscopic evaluation. **Results:** The most active extract was methanol extract with IC_{50} 114,659 µg/mL for arginase inhibitory activity and IC_{50} 78.47 µg/mL for DPPH scavenging activity. The secondary metabolite of methanol extract presence compound of alkaloid, flavonoid, tannin, terpene, and glycoside. The total flavonoid content was 141.10 mg/gram extract. The star-shape trichoma was identified as a specific fragment. **Conclusion:** The methanol extract of *Sterculia macrophylla* showed activity as arginase inhibitor and antioxidant.

Key words: Arginase, Antioxidant, Flavonoid, Pharmacognostical, *Sterculia macrophylla*.

Rini Prastiwi[1,2], Berna Elya[1,*], Rani Sauriasari[3], Muhammad Hanafi[4], Yesi Desmiaty[5]

[1]Department of Pharmacognosy-Phytochemistry, Faculty of Pharmacy Universities Indonesia, Depok 16424, West Java, INDONESIA.
[2]Department of Pharmacognosy-Phytochemistry, Faculty of Pharmacy and Science Muhammadiyah Prof. Dr. Hamka University, 1340 Jakarta, INDONESIA.
[3]Department of Pharmacology, Faculty of Pharmacy Indonesia University, Depok 16424, West Java, INDONESIA.
[4]Research Center for Chemistry, Indonesian Institute of Science, Serpong (LIPI) INDONESIA.
[5]Department of Pharmacognosy-Phytochemistry, Faculty of Pharmacy Universitas Pancasila, Depok, West Java, INDONESIA.

Correspondence

Berna Elya

Department of Pharmacognosy-Phytochemistry, Faculty of Pharmacy Universitas Indonesia, Depok, West Java, INDONESIA.

Phone no : +62 21 7270031

E-mail: berna.elya@gmail.com

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INTRODUCTION

Endothelial cell dysfunction (ECD) is a generic term that indicates dysregulation of endothelial cell function, including a decreased function of endothelial cells, vasodilation, impaired proliferative capacity, angiogenic properties, impaired function, and prevention of white blood cells from adhesion. Some of the factors that contribute to this including smoking, high blood pressure, diabetes, high cholesterol, obesity, hyperglycemia and genetic factors.[1-5] The diseases due to endothelial function disorders are aging, diabetes, hypertension, arthritis, and atherosclerosis.[6-10] Arginase (arginine ureahydrolase, or amidinohydrolase) is the hydrolytic enzyme responsible for converting arginine into ornithine and urea.[4,11] Arginase plays an important role in nitrogen metabolism. The role of arginase activity in influence the balancing of NO production in the body makes arginase as one of the prospective therapeutic targets for endothelial disease disorders.[12-14] Previous literature was showed that apigenin, isovitexin and vitexin inhibit arginase less than 50%. Galangin and quercitrin 50-70% inhibition. While isoquercitrin, isoorientin, and orientin 70-75%. The best inhibition was fisetin (87%), luteolin (83%), quercetin (83%) and, 7.8-dihydroxyflavone (80%). Flavonoid Fisetin has a higher arginase inhibition capability of 4 x compared with quercetin and 10x compared with luteolin. The substitution of the hydroxyl group at position 3 increases the activity of the arginase inhibitor, while in position five is not. Quercetin 2x is more potent than luteolin because of substitution at number 3 by hydroxyl groups.[15] *Scutellaria indica* (family *Labiatae*) Flavonoids compounds are 5,7-dihydroxy-8,20-di-methoxyflavanone and 5,20,50-trihydroxy-7,8-dimethoxy-flavanone, exhibiting inhibitory activity of each arginase of IC_{50} 25 , 1 (µM) and 11.6 (µM), while for positive control with Piceatannol-30-ObD-glucopyranoside (PG) of 1.0 (µM).[16] *Cecropia pachystachya* compound Chlorogenic acid, catechin, epicatechin and isoquercitrin can inhibit above 50% at 20 µM. Orientin IC50 is 16 µM, while IC_{50} for ethyl acetate and methanol soluble containing approximately 24% content orientin of 48 µg / mL.[17] *Saururus chinensis* content 7-Hydroxysauchinone compounds have IC_{50} of 89.6 µM while compound C-7 of sauchinone have arginase inhibition activity amounted to 61.4 µM.[18] The arginase inhibitor activity of the best of the three plants (*A. altilis, F. exasperata, K. africana*) showed that *A. altilis* at concentrations of 500 and 750 mg / mL did not show significant differences with the positive control catechin. From the experiment showed that the highest levels of flavonoids were in *A. altilis*[19] *Caesalpinia sappan* Lignum has activity as arginase inhibitor with IC_{50} 36.82µg / ml.[7]

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Based on literature study found that flavonoid compound has an activity of arginase inhibitor. It becomes attractively researched.

One genus of plants that contain flavonoid compounds is the genus of *Sterculia*. *Sterculia macrophylla* Vent. is a native plant of Indonesia, which many encountered in the territory of Indonesia. The literature study confirms that the most natural product of the genus *Sterculia* is flavonoids. These results also confirm that flavonoid plays a significant role in chemotaxis. Flavonoids in *S. macrophylla* are flavones, flavones C-glycosides, flavonols, flavans, isoflavones, isoflavan, and anthocyanins. The phenolic compounds such as phenolic acids, phenyl propanoid, coumarin, lignans and lignin content far less than the flavonoids.[20] The other metabolites in the genus of *Sterculia* include terpenoids, steroids, alkaloids, sugars and fatty acids. Wood, leaves, fruits, seeds and roots from *Sterculia* species traditionally widely used in various countries for treating various diseases, including gastrointestinal diseases, diabetes, respiratory-related diseases and skin diseases. Also, based on studies of the genus *Sterculia* has been studied and has activity as antimicrobial, anti-inflammatory, antioxidant, anticancer[20] and malaria.[21]

S. macrophylla was used as an aphrodisiac in Java.[22] This aphrodisiac effect could be related to arginase inhibitory activity because the increase of arginase activity has been implicated in erectile dysfunction.[23] Based on our literature study, pharmacognostical, arginase inhibitory effect and antioxidant activity of *Sterculia macrophylla* leaves have never been studied before. For this reason, the present work is design to investigate pharmacognostical, and to evaluate arginase inhibitory effect and antioxidant activity of *S. macrophylla* leaves.

MATERIALS AND METHODS

Materials

Sterculia macrophylla Vent. plant leaves. Collected in February 2017 from Botanical Garden of Bogor and determined in **Botany Herbarium Research Institute, Cibinong, West Java**. The solvents used in the study were n-hexane, ethyl acetate, and methanol purchased from local suppliers. KLT plate (Merck, Germany), silica gel 60 (Merck, Germany), aqua bidestilata, arginase enzymes (Sigma, Singapore), nor-NOHA standard (Cayman, USA), L-arginine (Sigma, Singapore), maleic acid (Sigma, Singapore), manganese sulfate (Sigma, Singapore), urea assay kits (Quantichrom® Bioassay, United States), dimethyl sulfoxide (Merck, Germany), methanol pro analysis (Merck, Germany), ethyl acetate pro-analysis (Merck, Germany), n-hexane pro analysis (Merck, Germany), proliferated pro analysis (Merck, Germany). Other ingredients for identification of the compound and the identification of powdered microscopic.

Macroscopic and microscopic identification

Leaves are identified in macroscopic shape, margins, color, tip, a base of the leaves. Identification of the microscopic was done on the leaf powder to know the fragment of the identifier. Studies of morphological plants were observed based on the description given in Evans WC and Indonesian Herb Pharmacopoeia.[24-25] Organoleptic characters were observed, noted and photographs were taken in the original environment.

Extraction

The extraction was done by maceration method using n-hexane, ethyl acetate, and methanol solvent. Preliminary extraction for screening was done with 20 g of leaf powder with solvent 200 mL. The extract was dried with a vacuum of a rotary evaporator at a temperature of 50°C and then continued to dry in water bath a temperature of 50°C. The extract obtained tested the activity of arginase inhibitor.

Preparation of extracts

The initial test of arginase inhibition activity of the three extracts using a concentration of 100 ppm. Extract *S. macrophylla* (50 mg) was added DMSO 400 µL shake until dissolved and added with aqua bidestillata to 5.0 ml. Then 1 ml of this solution was put in the vortex tube and added with aqua bidestillata up to 2 mL. 90 µL was taken into the micro tube and was added by aqua bidestillata up to 1 mL. 10 µL of this solution was added to each sample. The variation concentration to determined IC_{50} are 10, 40, 70, 100,130, and 160 ppm.

Arginase activity

Identify of the arginase inhibitory activity by using the procedure contained in the kit with slight modification. Ten (10) µL samples (extract), 15 µL enzyme solution, and 25 µL L-arginine solution were added to the well, shake. The mixture was incubated for 30 min at 37°C, then each sample was added by mixture of reagents A and B (100 µL) from the urea kit, shake and incubated at room temperature for 60 min. The result was measured at 430 nm. The positive control was performed under the same conditions. nor-NOHA used as positive control. The experiments were performed in triplicate.

Antioxidant activity

Antioxidant activity of methanol extract was evaluated using DPPH scavenging ability assay, which was conducted in a 96-well plate according to previously used method Zhang lu *et al.*[26] with slight modification. Samples in different concentrations (100, 250, 500, 1000, 1500, 2000 ppm) and 0.114 mM DPPH solution 180 µL in methanol were added to each well. Absorbance at 517 nm was read after 30 min of reaction in the dark with a micro-plate reader. The scavenging ability (%) was calculated as follows:

$$\text{Scavenging ability (\%)} = \text{x } 100\%$$

AC was the absorbance of control (without sample), AS is the absorbance of sample. Ascorbic acid was used as positive standard. All tests were performed in triplicate. The concentration of samples resulting in 50% inhibition on DPPH (IC_{50} value) were calculated.

Determination of total flavonoids

Determination of flavonoids content was measured on extracts that had the highest inhibition. The method by Chang *et al.* with slight modification.[27] Extract (200 mg) added ethanol 96% pa to 25 ml. From this sample solution taken 0.5 mL, then added with 1.5 ml of ethanol 96% pa, $AlCl_3$ p 10%, 0.1 mL, Na-acetate 1M, and, 2.8 mL aqua destilata. The total volume of the solution is 5 mL. Shake this solution and then incubated at room temperature for 30 min. The absorbance of this solution measurement using UV-Vis spectrophotometer at 437 nm. The control used is quercetin standard.

Phytochemical screening

The identification of content compound in the extract were carried out on the most active extract (methanol). The extracts was subjected to preliminary phytochemical investigation for the detection of following compounds; terpenoids, glycosides, flavonoids, alkaloids, tannins, anthraquinones, and saponins. The procedures described by Indonesian Herb Pharmacopoeia and Harborne.[28]

Chromatographic Profile

Thin layer chromatographic (TLC) profile evaluated of *S. macrophylla* leaves extract (methanol) using silica gel 60 F254 TLC plates for the chromatographic profile. The extract was dissolved in methanol. The mobile phases for the plates developed were chloroform-acetone-

formic acid (7:2:1). The plates were dried and observed under visible light and ultraviolet light 366 nm, and by spraying with 10% $AlCl_3$. The retention factor (Rf) value was calculated.[28]

RESULT

Microscopic and macroscopic

Leaves are green, slightly bitter and having characteristic odor. These are ovate shape, pinnatifid, acuminate apex, crenate, cordate base and 25–40cm in length, 20–30 cm in width. The microscopic and macroscopic fragment were show in Figure 1.

Determination of flavonoid levels

Quercetin levels were calculated as total flavonoid levels in the sample. From the calculation of quercetin standard curve, there is a linear correlation between absorbance and concentration with linear regression equation y = 0, 02448 x - 0, 2847 and value of relation coefficient (r) = 0, 9999. Based on the measurement results, the average value of flavonoids in each gram of extract was 141.10 mg ± 0.712.

Arginase activity

The preliminary study on arginase activity of three extracts. Based on the results obtained the active extract is methanol. The study of arginase activity performed on a methanol extract obtained IC_{50} 97.25 ppm, while the value of IC_{50} for nor-NOHA as a positive control is 3.77 ppm. The result on arginase activity showed in Table 1.

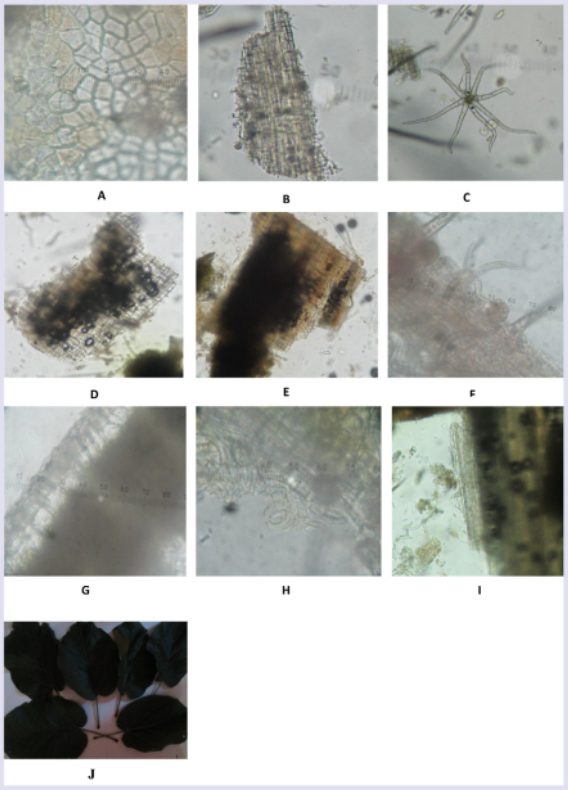

Figure 1: Macroscopic and Microscopic of *S. macrophylla* leaves powder. A= Pharenchym, B= Parenchym, C= Star shape trichoma, D= Parenchym, E= Cortex, F= Star shape trichoma on parenchym, G= Epidermis, H= Stomata, I= Phloem, J= Leaves *S. macrophylla*.

Antioxidant activity

The antioxidant activity test was performed using DPPH assay. The following results are obtained. The result of antioxidant activity on the Table 2.

The IC_{50} of methanol extract was 78, 47 ppm, while vitamin C as a positive control was 2.23 ppm.

Phytochemical screening

Phytochemical screening showed presence of flavonoids, glycosides, alkaloids, tannins, terpenes, and saponins and negative to antraquinone. Here is the results in Table 3.

Chromatographic Profile

The chromatographic profile had 9 spots, and the spot no 4 (Rf 0.43) have Rf value with standard quercetin. The result was displayed in Table 4.

Table 1: Average inhibition arginase of extract methanol.

Concentration (ppm)	Average inhibition (%)	SD	IC_{50} (ppm)
10	25,4985	±2,79	
70	34,9908	±0,99	
100	44,5485	±2,95	97,25
130	57,7809	±10,69	
160	81,9962	±2,11	
Nor-NOHA			3,77

Table 2: Antioxidant activity of extract methanol.

Concentration (ppm)	Antioxidant activity	Sd	IC_{50}
10	11,6602	±2,13	
25	19,7806	±3,53	
50	43,3889	±3,19	78,47
100	78,6366	±2,82	
150	85,5816	±0,27	
200	88,3613	±1,84	
Vitamin C			2,23

Table 3: Phytochemical screening of extract methanol.

Chemical Constituent	Result
Alkaloids	+
Flavonoids	+
Terpens	+
Tannins	+
Glycosides	+
Anthraquinones	-

Note + = Presence
= Absence

Table 4: Chromatographic profile of extract methanol.

Extract	Solvent system	Total spot	Rf	UV365	$AlCl_3$ 10%
Methanol extract	Chloroform: acetone:formic acid	9	Rf 1 = 0.1	Rf 1 = light blue	Rf 3 = yellow
			Rf 2 = 0.21	Rf 2 = yellow	Rf 4 = yellow
			Rf 3 = 0.35	Rf 3 = yellow	Rf 5 = yellow
			Rf 4 = 0.43	Rf 4 = yellow	
			Rf 5 = 0.5	Rf 5 = light blue Rf 6= red	
			Rf 6= 0.53	Rf 7 = red	
			Rf 7 = 0.7	Rf 8 = red	
			Rf 8 = 0.9	quercetin = yellow	quercetin = yellow
			quercetin = 0.43		

DISCUSSION

Phytochemical screening result showed that The methanol extracts contain flavonoids, glycosides, alkaloids, tannins, terpenes and saponins. Flavonoids were known have arginase inbitory activity.[15] The high content of flavonoid compounds in methanol extract provides the possibility that these flavonoids have a role as an arginase inhibitor. The catechol group in flavonoid possibly interacts with one of arginase site such as Asp 129.[29-30] From the chromatographic profile, we can see that methanol extract of *S. macrophylla* positively contain quercetin, one of arginase inhibitor.[15] In the preliminary experiment, the most active extract was methanol extract. The inhibitory potency of methanol extract against arginase II activity is lower than nor-NOHA, but with IC50 114,659 µg/mL is a natural moiety from natural medicinal plants that inhibit arginase activity would be useful for the development of pharmaceutical natural compounds. Antioxidants can inhibit free radicals and prevent from various degenerative diseases. The ability to donate electrons from natural materials can be measured by decolorization of the DPPH solution. The method is based on scavenging of DPPH through the addition of a radical species. The rate of color change is proportional to the concentration and potency of antioxidants. A large reduction in the absorbance of the reaction mixture showed significant free radical scavenging activity on the tested compound.[19] NO is a free radical with a short half-life (<30 s) and its independent action may cause neuronal damage, especially in conjunction other ROS such as superoxide radical to form peroxynitrite radical.[31] This study exhibited that methanol extract of *S. macrophylla* has good arginase inhibitory activity and antioxidant activity.

CONCLUSION

The methanol extract has IC_{50} 114,659 µg/mL for arginase inhibitory activity and IC_{50} 78.47 µg/mL for DPPH scavenging activity. The secondary metabolite of methanol extract presence compound of alkaloids, flavonoids, tannins, terpenes, and glycosides. The total flavonoid content was 141.10 mg/gram extract. The star-shape trichoma was identified as a specific fragment.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

ACKNOWLEDGEMENT

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ABBREVIATIONS

N-hidroksi-nor-L-arginin: nor-NOHA; *Sterculia macrophylla*: *S. macrophylla*.

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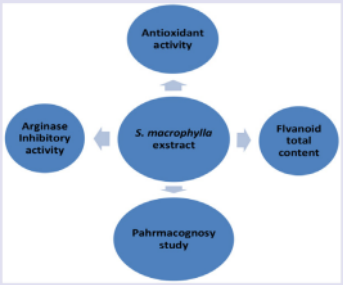
GRAPHICAL ABSTRACT



SUMMARY

- *Sterculia macrophylla* Vent. Leaves. has arginase inhibitory activity and antioxidant activity. The pharmacognosy study of *Sterculia macrophylla* Vent. Leaves. provides useful information for quality control parameters for the crude.

ABOUT AUTHORS

Rini Prastiwi: Lecturer and researcher at Faculty of Pharmacy and Sciences, University of Muhammadiyah Prof. Dr. Hamka, Klender, Jakarta 13460, Indonesia, and student at Faculty of Pharmacy Universitas, Indonesia.

Berna Elya: Lecturer and researcher at Faculty of Pharmacy Universities, Indonesia, Depok 16424, West Java, Indonesia.

Rani Sauriasari: Lecturer and researcher at Faculty of Pharmacy Universities Indonesia, Depok 16424, West Java, Indonesia.

Muhammad Hanafi: Researcher at Research Center for Chemistry, Indonesian Institute of Science, Serpong, (LIPI) Indonseia and lecturer at Faculty of Pharmacy Universitas Pancasila, Depok, West Java, Indonesia.

Yesi Desmiaty: Lecturer and researcher at Faculty of Pharmacy Universitas Pancasila, Depok, West Java, Indonseia and student at Faculty of Pharmacy Universitas, Indonesia.

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