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Original Article

Arginase Inhibitory Activity and Total Flavonoid Content on *Caesalpinia ferrea* C. Mart Stem Bark Extracts

Devi Indriani, Berna Elya*, Arikadia Noviani

ABSTRACT

Background: Flavonoids, polyphenolic compounds that are ubiquitous in nature, have been known for their pharmacological as antifungal, diuretic, antihistamin, antihypertension, insecticide, bactericide, antiviral, antioxidant, and enzim inhibitor. Flavanones found in all parts *Scutellaria indica*, has the ability to inhibit arginase, flavanols found in the seeds of *Theobroma cacao* L. Previous study showed that *Caesalpinia ferrea* C. Mart stem bark contains flavonoid compound. **Objective:** The objective of this study to analyze arginase inhibitory activity and to determine the total flavonoid content of *Caesalpinia ferrea* C. Mart stem bark by using $AlCl_3$ colorimetric method. **Methods:** Dried *Caesalpinia ferrea* stem barks were refluxed with three different solvent with gradual gradient polarity i.e *n*-hexane, ethyl acetate, and methanol. Each extract was tested to determine arginase inhibitory activity. Total flavonoid content was determined on extract showed the highest arginase inhibitory activity. **Results:** Methanolic extract showed arginase inhibitory activity of 12.81% and flavonoid content was 2 mgQE/g. Phytochemical screening on *Caesalpinia ferrea* stem bark ethyl acetate extract showed that it contains flavonoids, tannins, saponins, steroids, and terpenoids, meanwhile *Caesalpinia ferrea* stem bark methanolic extract contains flavonoids, tannins, saponins, and steroids. **Conclusion:** *Caesalpinia ferrea* C. Mart stem bark extracts were not potential to inhibit arginase.
Key words: Arginase, *Caesalpinia ferrea* C. Mart, Flavonoids.

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INTRODUCTION

Polyphenols, a secondary metabolite found in fruits, vegetables, and herbs, are widely used to treat chronic diseases such as cardiovascular disease, obesity, diabetes and cancer.[1] The compound has the ability to increase the activity of NOS (nitric oxide synthase). Increasing the production of NO (nitric oxide) by inbiting the arginase enzymes in mammals.[2] The increased NO (nitric oxide) in blood vessel cells has an important role in inhibiting platelet aggregation, leukocyte adhesion and infiltration into blood vessel walls, and proliferation of smooth muscle cells of blood vessels.[3] Previous study showed some active compounds from plants having arginase inhibitory activity that are polyphenols[4] such as chlorogenic acid, piceatannol, resveratrol, and flavonoids.[5]

Flavonoids are one of the polyphenolic compounds that widely found in almost every green plant. Flavonoids structure of flavonoids has 15 carbon atoms consisting two aromatic rings (ring A and ring B) connected with three carbon units.[6] Flavonoids exist in all parts of the plant such as leaves, roots, wood, bark, flowers, fruits, and seeds. Pharmacological studies of several flavonoid compounds have shown their ability to serve as antifungal, diuretic, antihistamine, antihypertensive, insecticides, bactericidal, antiviral, antioxidant, and inhibit enzymes.[7]

Flavonoid compounds has the ability to inactivate enzymes by hydrolizing glycosides into active aglycons.[8] Flavanones found in all parts *Scutellaria indica*,[9] has the ability to inhibit arginase, flavanols found in the seeds of *Theobroma cacao* L.,[10] quercetin, and kuersitrin.[11] More hydroxyl groups in the B ring flavonol, can increased activity of arginase inhibition.[12]

Arginase inhibitor compounds include boric acid derivatives (S- (2-boronoethyl) -L-cysteine (BEC) and 2- (S) -amino-6-boronohexanoic acid (ABH)) and one of arginine analog compound that is N (omega) -hydroxy-nor-L-arginine (nor-NOHA). However, BEC and ABH are potentially toxic and have some pharmacokinetic problems.[13] As for nor-NOHA has a very short half-life.[14] Thus, a better compound to serve as arginase inhibitor is required. It expected to foundant arginase inhibitors from natural ingredients.[15]

Studies about arginase inhibitory activity had been performed on ethyl acetate extract from *Caesalpinia sappan* showing effect of inhibition with IC_{50} value equal to 36.82 µg / mL.[16] Other studies showed that *Caesalpinia ferrea* C. Mart contains flavonoids, saponins, tannins, coumarin, steroids and phenolic compounds.[17] Some of the pharmacological activities known from *Caesalpinia ferrea* extracts, anti-inflam-

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matory activity,[18] antimicrobial,[19] cancer chemopreventive,[20] antiarrhythmics, are vasoleraksan[21] and also used for peptic ulcers treatment.[22]

Thus, based on above description, this study aim to conduct arginase inhibitory activity test and determination of total flavonoid content on *Caesalpinia ferrea* C. Mart stem bark extracts.

MATERIALS AND METHODS

Materials

This study used the stem bark of *Caesalpinia ferrea* C. Mart obtained from the Center for Conservation of Plants- Bogor Botanical Gardens, nor-NOHA acetate (Cayman, USA), quercetin (Sigma Aldrich, India), arginase, L-arginine, maleic acid, $MnSO_4$ (Sigma Aldrich, Singapore), urea kit assay (Abnova, Taiwan), solvents, total flavonoid content determination reagents, and phytochemical screening reagents.

Preparation of *Caesalpinia ferrea* C. Mart stem bark extract

Stem bark of *Caesalpinia ferrea* C. Mart was collected in December 2016, obtained and identified by the Center for Conservation of Plants- Bogor Botanical Gardens.

Extraction

Dried powdered of stem bark (150 g) was extracted by using reflux method using three solvent having gradual polarity i.e*n*-hexane, ethyl acetate, and methanol, then evaporated.

Determination Arginase Inhibitory Activity

The determination of arginase inhibitory activity was determined by using methods made by Sigma Aldrich with some modification in subtrate and enzyme concentration. Mixture of 15µL of arginase 1 U/mL, 20 µLof L-arginine 570 mM, and 10 µL of sample solution were incubated at 37° C for 30 min. After preincubating, 100 µL of kit urea assay was added and incubated at room temperature for an hour. Arginase activity was determined by microplate reader (Epoch, USA) at λ 430 nm by measuring the quantity of urea released from arginase. Each sampels were made blank with added the enzyme after kit urea assay. Nor-NOHA was used as a positive control of arginase inhibitor. The arginase inhibitory activity was defined as IC_{50} value.

Determination of Total Flavonoid Content

Determination of total flavonoid content was conducted on extract having highest arginase inhibition. The method refers to the second method listed in Pharmacopoeia Herbal Indonesia Suplement II. Quersetin was used as standart is used to make calibration curves with final concentrations 3, 4, 5, 6, 7 and 8 µg/mL in ethanol pro analysis. As much as 0.5 mL sample solution of quercetin or extract was added to test tube, then 1.5 mL ethanol pro analysis was added; 0.1 mL $AlCl_3$10%; 0.1 mL of 1 M sodium acatate and 2.8 mL of distillate water. The volume of $AlCl_3$ 10% was replaced by the same quantity of the same volume as the sample blank. The mixture was centrifugate and incubated at room temperature for 30 min. The absorbance was measured by using a UV-Vis spectrophotometer (Jazco) at λ 437,5 nm. The total flavonoid content on extract was calculated by using y=a+bx,so the highest arginase inhibitioncan be calculated.

Phytochemical Screening

The phytochemical screening consited of alkaloids test (using mayer, dragendroff, and wagner reagents), flavonoids test with willstatter reaction, tannins test with gelatin test and ferrous (III) chloride, saponins test with honeycomb froth test, quinones with NaOH, steroids and triterpenoids with libermann-burchard reagent was performed on ethyl acetate and methanol extract.

RESULTS AND DISCUSSION

Extraction

The obtained extracts (Table 1) were tested to measure the arginase inhibitory activity and determination of total flavonoid content was conducted on extract having highest arginase inhibition.

Determination *Arginase Inhibitory Activity*

The IC_{50} value of nor-NOHA acetate is 3,7749 µg/mL (Table 2). The IC_{50} value of nor-NOHA acetate towards arginase from mouse macrophages listed on product info is 10-12 µM or is 3.556 µg/mL. This suggested that the nor-NOHA acetate's arginase inhibitory activity had no significant differece compared to previous studies as noted in the product info.

The *n*-hexane and ethyl acetate extract had no inhibitory effect on the arginase enzyme shown as negative result (Table 3). Table 3 showed that methanol extract had the highest arginase inhibitory activity that was 12.81%. These results showed a low ability to inhibit arginase due to the less active of the compound, therefore further IC_{50} calculation was ommited.

Determination of Total Flavonoid Content

The methanol extract of *Caesalpinia ferrea* stem bark absorbance was plotted in quercetin calibration curve then the total flavonoid content was calculated. The total flavonoid content was expressed in QE (Quercetin equivalent) which is the amount of milligram equivalent of quercetin in 1 g of sample. Based on the results, in 1 g of methanol extract stem bark *Caesalpinia ferrea* contained 2 mg equivalent quercetin.

Phytochemical Screening

Phytochemical screening result is showed in Table 4.

Table 1: The result of extraction by maceration method.

Extracts	Results (gr)	Results (%)
n-Hexane	0.87	0.58
Ethyl acetate	4.93	3.29
Methanol	13.47	8.98

Table 2: The IC_{50} of nor-NOHA acetate.

Concentration (µg/mL)	Percent Inhibition (%)± SEM	IC_{50}(µg/mL)
1	33.90 ± 2.860	3.7749
3	49.20 ± 8.914	
4	50.43 ± 7.304	
5	55.39 ± 9.638	

Table 3: The IC_{50} value of n-hexane, ethyl acetate and methanol extract of *Caesalpinia ferrea* C. Mart Stem Bark.

No.	Extract (100 µg/mL)	Percent Inhibition (%)± SEM
1	*n*- Hexane	-37.87 ± 7.439
2	Ethyl acetate	-23.87 ± 2.236
3	Methanol	12.81 ± 0.890

Table 4: Phytochemical screening of ethyl acetate and methanol extract.

Phytochemical Contents	Ethyl acetate extract	Methanol extract
Flavonoid	+	+
Alkaloid	-	-
Tannin	+	+
Saponin	+	+
Quinone	-	-
Steroid	+	+
Triterpenoid	+	-

CONCLUSION

Methanolic extract showed 12.81% arginase inhibitory activity in 100 µg/mL concentration and contained 2 mgQE/g flavonoid content. This showed that *Caesalpinia ferrea* C. Mart stem bark extracts were not potential to inhibit arginase. Phytochemical screening result showed that *Caesalpinia ferrea* stem bark ethyl acetate extract contained flavonoids, tannins, saponins, steroids, and terpenoids, meanwhile *Caesalpinia ferrea* stem bark methanolic extract contained flavonoids, tannins, saponins, and steroids.

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CONFLICT OF INTEREST

The authors declare no conflict of intetrest.

ABBREVIATIONS

nor-NOHA acetate: N(omega)-hydroxy-nor-L-arginine acetate; **AlCl3:** Alumunium chloride; **QE:** Quercertin Equivalent; **NOS:** Nitric Oxide Synthase; **NO:** Nitric Oxide; **BEC:** (S-(2-boronoethyl)L-cysteine); **ABH:** 2-(S)-amino-6-boronohexanoic acid; **UV:** Ultra Violet; **MnSO4:** Manganese (II) Sulfate; **NaOH:** Sodium Hydroxide.

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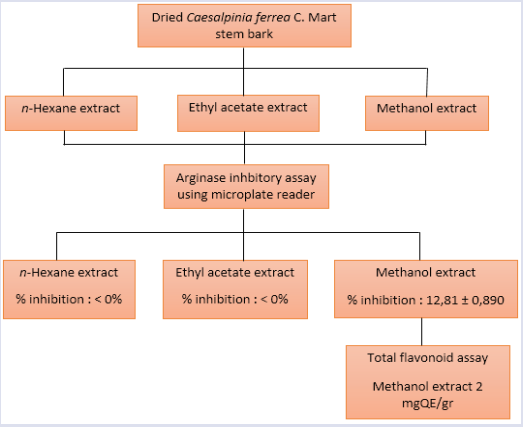
GRAPHICAL ABSTRACT



SUMMARY

- *Caesalpinia ferrea* C. Mart stem bark were refluxed successively with n-hexane, ethyl acetate, and methanol.
- Methanolic extract showed 12.81% arginase inhibitory activity in 100 µg/mL concentration and contained 2 mgQE/g flavonoid content.
- *Caesalpinia ferrea* C. Mart stem bark extracts were not potential to inhibit arginase.

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