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

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ABSTRACT

Background: Flavonoids, polyphenolic compounds that are ubiquitous in nature, have been known for their pharmacological as antifungal, diuretic, antimicrobial, antihypertension, insecticide, bactericide, antiviral, antioxidant, and enzim inhibitor. Flavanones found in all parts Scutellaria indica, has the ability to inhibit arginase, flavonols 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 AlCl3 colorimetric method. Methods: Dried Caesalpinia ferrea stem barks were refluxed with three different solvent with gradual gradient polarity i.e. 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.

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 inhibiting the arginase enzymes in mammals. 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. Previous study showed some active compounds from plants having arginase inhibitory activity that are polyphenols such as chlorogenic acid, piceatannol, resveratrol, and flavonoids. 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. 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, antimicrobial, antihypertensive, insecticides, bactericidal, antiviral, antioxidant, and inhibit enzymes.

Flavonoid compounds have the ability to inactivate enzymes by hydrolyzing glycosides into active aglycons. Flavanones found in all parts Scutellaria indica, has the ability to inhibit arginase, flavonols found in the seeds of Theobroma cacao L., quercetin, and kuersitrin. More hydroxyl groups in the B ring flavonol, can increased activity of arginase inhibition. 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). 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. As for nor-NOHA has a very short half-life. Thus, a better compound to serve as arginase inhibitor is required. It expected to foundant arginase inhibitors from natural ingredients.

Studies about arginase inhibitory activity had been performed on ethyl acetate extract from Caesalpinia sappan showing effect of inhibition with IC50 value equal to 36.82 μg / mL. Other studies showed that Caesalpinia ferrea C. Mart contains flavonoids, saponins, tannins, coumarin, steroids and phenolic compounds. Some of the pharmacological activities known from Caesalpinia ferrea extracts, anti-inflamm-
matory activity, antimicrobial, cancer chemopreventive, antiarrhythmics, are vasoleraksan and also used for peptic ulcers treatment. 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, MnSO₄, maleic acid, S.aureus (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. 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 substrate and enzyme concentration. Mixture of 15 µL of arginase 1 U/mL, 20 µL of 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 samples 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 IC₅₀ 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. Quercetin 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 AlCl₃ 10%; 0.1 mL of 1 M sodium acetate and 2.8 mL of distilled water. The volume of AlCl₃ 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 inhibition can be calculated.

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 IC₅₀ value of nor-NOHA acetate is 3.7749 µg/mL (Table 2). The IC₅₀ 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 differerce 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 IC₅₀ 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.
**SUMMARY**

Cocoa flavanols lower vascular arginase activity in human endothelial cells. Methanol 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 interest.

**ABBREVIATIONS**

nor-NOHA acetate: N(omega)-hydroxy-nor-L-arginine acetate; AlCl3: Aluminium chloride; QE: Quercetin 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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**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, while *Caesalpinia ferrea* stem bark methanolic extract contained flavonoids, tannins, saponins, and steroids.

**GRAPHICAL ABSTRACT**

- 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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