Inhibitory Effect on Arginase and Total Phenolic Content Determination of Extracts from Different parts of *Melastoma malabathricum* L.

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**ABSTRACT**

**Background:** Some phenolic compounds are potential source for arginase inhibitor that can be used in management of disease associated with endothelial dysfunction. **Objective:** The aim of this study was to investigate the inhibitory effect on arginase and to determine total phenolic content of extracts from different parts of *Melastoma malabathricum* L. **Methods:** Each part were extracted with 70% ethanol by maceration. The extracts were mixed with bovine liver arginase and L-arginine as a substrate. The inhibitory effect of extracts were determined *in vitro*, by measuring its absorbance on 430 nm. Total phenolic content were determined with Folin-Ciocalteu 25% on 750 nm. **Results:** The 70% ethanol extract of *M. malabathricum* leaves at 100 µg/mL found to have highest inhibition (81.26 ± 5.27 %) followed by flower leaves at 100 µg/mL was found to have highest inhibition (73.39 ± 9.39%), fruit 67.63 ± 7.61 and stem 61.61 ± 4.56%. The IC50 value of the most active extract was 62.43 µg/mL while the IC50 value of N-hidroksi-nor-L-arginin (nor-NOHA) acetate, an arginase inhibitor was found to be 3.91 µg/mL. Total phenolic content of 70% ethanolic extracts of *M. malabathricum* leaves, flower, fruit and stem was 15.9, 20.7, 6.44, and 6.29%. **Conclusion:** The 70% ethanol extract of *M. malabathricum* leaves exhibited the highest inhibition on arginase but highest total phenolic content was from flower part. Pearson correlation test showed no correlation p>0.05 between arginase inhibition and total phenolic content of the samples.

**Key words:** Arginase, Larginine, *Melastoma malabathricum*.

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**INTRODUCTION**

Polyphenols are one of secondary metabolite in plants that have beneficial role on protective effect of cardiovascular system including improve endothelial function. 1 Endothelial dysfunction is primarily due to reduction in Nitric Oxide (NO) bioavailability, impairment of endothelium-dependent vasorelaxation caused by a loss of nitric oxide bioactivity in the vessel wall. 2 Endothelial dysfunction can result from and/or contribute to several disease development, as occurred in hypertension, diabetes mellitus and hypercholesterolemia. 1, 3 Arginase is manganese metalloenzyme. 3 Upregulation of Arginase can reduce the bioavailability of NO through competition between arginase with Nitric Oxide Synthase (NOS) in using L-arginine as substrate, which can cause endothelial dysfunction. 1, 4, 5 Potent inhibitor arginase such as S-(2-borononyethyl)-L-cysteine (BEC), 2-(S)-amino-6-boronohexanoic acid (ABH) and (omega)-hydroxy-nor-L-arginine (nor-NOHA) have some problem to be more developed. BEC and ABH might confer a potential toxicity and pharmacokinetic problem due to their boronic acid functionality. 4 On the other hand, nor-NOHA have very short half-life. 4 According to this fact, it is necessary to find arginase inhibitor from natural sources. Until now, study on arginase inhibitor from plants was still limited.

Previous study showed that phenolic compounds such as quercetin, kaempferol, epicatechin has inhibitory effect on bovine arginase with IC50 31.2, 179.1, and 19.9 µg/mL.1 *Melastoma malabathricum* containing those three phenolic compounds1 and other phenolic compounds so we hypothesized that extracts from different plant of this plant may have arginase inhibitory effects. The plant were also chosen based on their pharmacological effect on patogenesis some diseases related to upregulation of arginase such as hypertension, 2-4 hyperlipidemia 5 and wound healing. 6 To test this hypotesis, we investigated the inhibitory effect on arginase of different parts of this plant using *in vitro* methods. According to literature search, inhibitory effect on arginase from these different parts of this plants have never been studied. In order to see the relationship between arginase inhibitory effect and polyphenolic content, total phenolic content of each extract was also determined.

**MATERIALS AND METHODS**

**Chemicals**
	nor-NOHA acetate (Cayman, US), gallic acid (Sigma Aldrich, India),
aqua bidestilata, bovine liver arginase (Sigma, Singapore), foline-ciocalteu (Merck, Germany), L-arginine (Sigma, Singapore), maleic acid (Sigma, Singapore), mangan sulfate (Sigma, Singapore), urea assay kit (Abnova, Taiwan), dimethyl sulfoxide (Merck, Germany).

**Plant materials**

Parts of *M. malabathricum* were collected in July 2017 from central of Kalimantan. Plants were identified by Indonesia Science Institution, Center for Plant Conservation-Bogor Botanical Garden, Indonesia.

**Preparation of samples**

The part of the plant were collected and cleaned, dried at room temperature, and then powdered and stored in an air tight glass container. 50 g of each plant part was extracted by maceration with 500 mL of 70% ethanol...
as the solvent in first day at room temperature, and continued macerated with 250 mL of 70% ethanol in second until fourth day. The extract then evaporated using a rotatory vacuum evaporator, and then dried in water bath on 50°C.

Determination of percent yield (%)
The extraction yield was calculated for each extract using the formula below:

\[ \text{Percent yield} = \frac{a}{b} \times 100 \]

(a) is dry weight of extract and (b) soaked samples material

Arginase inhibition assay
The inhibition of arginase activity was performed using the enzyme protocol (Sigma Aldrich)\(^{11}\) and urea assay kit obtained from Abnova Corporation, Taiwan (KA 1652)\(^{12}\) with slight modification. N-hidroksi-nor-L-arginin (nor-NOHA) acetate was used as the standard inhibitor. Briefly, 10 µL of the 100 µg/ml extract solution, 15 µL of arginase solution (1 U/mL) and 20 µL L-arginine 570 mM as the substrate of arginase, was added into the well. The mixture were shaken and incubated for 30 min at 37°C to have the complete reaction. In control wells the extract was replaced by dimethyl sulfoxide. The reaction was stopped by addition of 100 µL of urea assay kit (kit A : kit B = 1 : 1) into each well. The absorbance of urea was measured at 430 nm after incubation in room temperature 25°C for 60 min, by a microplate reader (Versamax microplate reader, USA).

Percent of inhibition was calculated using the formula:

\[ \% \text{Inhibition} = \frac{[\text{Abs1} - \text{Abs2}] - [\text{Abs3} - \text{Abs4}]}{[\text{Abs1} - \text{Abs2}]} \times 100 \]

Abs1 is the absorbance of the control, Abs2 is the absorbance of the control blank, Abs3 is the absorbance of sample, and Abs4 is the absorbance of sample blank. IC\(_{50}\) value represents the concentration of inhibitor required to achieve 50% of enzyme inhibition.

Phytochemical screening
The qualitative phytochemistry test were conducted according to Indonesian Materia Medica\(^{13}\) and Harborne.\(^{14}\) Alkaloid test with Mayer, Dragendorff, and Bouchardat reagents; flavonoid test with Shinoda and Wilson Tâuböck reaction; tannin test with gelatin test, gelatin-salt test, and test with ferrous (III) chloride; saponin test with foaming test; terpenoid test with Liebermann-Burchard reagent.

Determination of total phenolic content
Total phenolic content was determined using the 96-well microplate with Folin–Ciocalteu reagent, adapted from Ahmad et al.\(^{15}\) with slight modification. 20 µL of the diluted extract or standard solution were added to 100 µL of reagent 25% Foline–Ciocalteu and homogenized by shaken for 60 s in 96-well microplate, and then allowed to stand for 4 min. After that, 75 µL of sodium carbonate solution (100 g/L) were added into the well and the mixture was shaken for 1 minute. After incubated for 2 h at room temperature, the absorbance was measured at 750 nm using the microplate reader (Versavax Microplate Reader). Extract or standard was substituted by ethanol as a blank. The standard curve for quantifying the phenolic contents was prepared by using gallic acid dilutions (6.25–200 mg/L). All samples were performed in triplicate.

RESULTS

The results showed that 70% ethanol extract of Melastoma malabathricum leaves gave the highest activity with percent inhibition of 81.26% ± 5.27 (Table 1) and IC\(_{50}\) 62.43 µg/mL with equation y = 0.8163x + 0.9606, R\(^2\) = 0.9876. The leaves of Melastoma malabathricum as appears in Figure 1. The extract was further investigated to determine the source of activity. The common phytochemistry content from plant such as flavonoid, alkaloid, terpenoid, steroid, tannin, and saponin have identified (Table 2).

### Table 1: In vitro arginase inhibition activity of different part of M. malabathricum.\(^{4}\)

<table>
<thead>
<tr>
<th>No.</th>
<th>Part of the plant</th>
<th>Percent yield (%)</th>
<th>Inhibition of Arginase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stem</td>
<td>8.44</td>
<td>61.61 ± 4.56</td>
</tr>
<tr>
<td>2</td>
<td>Fruit</td>
<td>13.84</td>
<td>67.63 ± 7.61</td>
</tr>
<tr>
<td>3</td>
<td>Flower</td>
<td>20.14</td>
<td>73.39 ± 9.39</td>
</tr>
<tr>
<td>4</td>
<td>Leaves</td>
<td>18.1</td>
<td>81.26 ± 5.27</td>
</tr>
</tbody>
</table>

Final concentration of the extract solution in the Arginase inhibition assay was 100 µg/ml.

Data are mean ± SEM for triplicate measurements.

**DISCUSSION**

Investigated samples were successively extracted by maceration method using 70% ethanol. Maceration method is cold extraction method by soaking raw materials for certain time in appropriate solvent. This method was chosen to avoid any degradation of any thermolabile active compounds during extraction which can caused by heat process.\(^{16,17}\) We used 70% ethanol as solvent due to the polarity of polyphenol as target compound.\(^{18}\) as studied before that some phenolic compounds has arginase inhibitory activity.\(^{1}\) Some polar and semi polar compounds could be extracted by 70% ethanol. The inhibitory effect of three 70% ethanol extracts on arginase activity was performed with in vitro method using L-arginine as substrate. Arginase will hydrolized L-arginine to L-ornithine and urea. Those condition will be accomplished by cleaving the guanidino group from arginine. It converts one positively charged...
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**Table 2: Phytochemical screening of the extracts.**

<table>
<thead>
<tr>
<th>Phytochemical Constituents</th>
<th>Stem</th>
<th>Fruit</th>
<th>Flower</th>
<th>Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Note: - = absent, + = present

**Table 3: Total phenolic content of the extracts**

<table>
<thead>
<tr>
<th>Part of the plant</th>
<th>Total Phenolic Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem</td>
<td>6.29</td>
</tr>
<tr>
<td>Fruit</td>
<td>6.44</td>
</tr>
<tr>
<td>Flower</td>
<td>20.7</td>
</tr>
<tr>
<td>Leaves</td>
<td>15.9</td>
</tr>
</tbody>
</table>

Data are mean ± SEM for triplicate measurements.

- It contains some phenolic compound such as quercetin, epicatechin, epicatechin galata, kaempferol which is suspected as responsible compound for inhibitor of mamalia arginase also cinnamic acid derivative which can be also responsible for the inhibitory effect on arginase. These compound worked synergistically in the extract and showed higher arginase inhibitory effect on arginase than other Part extract of the plant. In other study, flavonol and epicatechin from Cocoa showed inhibition of arginase in HUVEC, Flavanone from Scutellaria indica showed inhibition of arginase in mice kidney liseate. The result of total phenolic content of *M. malabathricum* flower extract showed highest phenolic content of this plant compared to other parts of plant extract. It can be considered that arginase inhibitor activity of this plant was not related with the high phenolic content. According to Thernier and Pudlo the catechol group suggested interacts with Asp 129 which is involved in metal bridge formation for the two Mn cofactors in the site of arginase.

**SUMMARY**

- Each part of *M. malabathricum* (flower, fruit, stem, leaves) were extracted by maceration using 70% ethanol as the solvent for 24 hours and repeated 3 times.
- The highest yield was flower (20.14%).
- Phytochemical screening showed that all of samples positively contain flavonoid, alkaloid, terpenoid, tannin.
- Total phenolic content was the highest in flower (20.7%) and the lowest in stem (6.29%).
- The highest inhibition on arginase was in leaf extract (81.26 ± 5.27%) followed by flower, fruit, and stem.

**REFERENCES**


