Inhibitory Effect of Lantana camara L., Eclipta prostrata (L.) L. and Cosmos caudatus Kunth. Leaf Extracts on ADP-Induced Platelet Aggregation

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ABSTRACT

Objective: The leaves of Lantana camara L., Eclipta prostrata (L.) L. and Cosmos caudatus Kunth. are rich in flavonoids, saponins and essential oils. This study was carried out to determine the antiplatelet effect of the three plant extracts on ADP-induced platelet aggregation. Methods: The samples consisted of clopidogrel as positive control, aquadest as negative control and test extracts with concentrations of 40, 60, 80 and 100 mg/ml. Platelet Rich Plasma (PRP) was added for each extract concentration. The absorbance test of the samples was measured at 600 nm, before and after the addition of 20 μL adenosine diphosphate (ADP) 5 mM. After ADP added, then measured again the plasma absorbance was measured of the same wavelength. Antiplatelet aggregation activity was determined by calculating plasma absorbance before and after addition of ADP. Results: The results showed that the inhibitory percentage of platelet aggregation with extract concentrations of 100, 80, 60 and 40 mg/ml were Lantana camara L. leaf extract (91.17%; 73.27%; 72.86% and 37.15%), Eclipta prostrata (L.) L. leaf extract (74.55%; 65.60%; 48.00% dan 39.08%) and Cosmos caudatus Kunth. Leaf extract (59.17%; 52.56%; 14.25% dan 13.82%), respectively. The test extracts inhibited in vitro ADP-induced platelet aggregation with IC50 value were as follows: 47.09 mg/ml (Lantana camara L), 59.02 mg/ml (Eclipta prostrata (L.) L.) and 87.26 mg/ml (Cosmos caudatus Kunth.). Conclusion: The present work demonstrated that the three extracts could inhibit in-vitro ADP-induced platelet aggregation and the best potency was shown by Lantana camara L leaf extract.

Key words: Anti-platelet aggregation, Cosmos caudatus Kunth., Eclipta prostrata (L.) L., Lantana camara L.

INTRODUCTION

Stroke is commonly caused by cardiovascular disorders such as atherosclerosis. Atherosclerosis is not a degenerative process, but it is a chronic inflammatory process followed by a process of reparation in the artery wall.1 The journey of atherosclerosis is histopathologically divided into several stages: 1) early lesions (fatty streak, with microthrombi), 2) lesions up (fibrosis, plaques of atheroma-atherosclerotic) and 3) the complicated lesions (ulceration, calcification, hemorrhage, gangrene, aneurysm and infarction). The complicated lesions occur on the process of thrombosis, platelet adhesion, platelet aggregation, and coagulation due to atheroma plaque rupture in arteries. Inside the plaque the process of coagulation occurs due to mixed blood with collagen. Coagulation factors such as factor VII activates factor X to occur, catalyze the conversion of prothrombin into thrombin to stabilize thrombus which then secrete platelet deposition and platelet derived growth factor (PDGF). Thrombus can undergo dissolution (lost) so that patients do not experience symptoms. Thrombus can also be attached to the process of atherosclerosis in which the blockage inside the artery lumen grows larger and cause clinical symptoms. Rupture or rupture of plaque followed by thrombus formation results in sudden lumen occlusion. Formed thrombus can cause a range of clinical symptoms because the thrombus goes along with the bloodstream and clog blood vessels distal to the size will be smaller. If the rupture is very large, then most likely there will be a blockage in the large blood vessels.1 The formation of such thrombus is medically preventable with antiplatelet aggregation agents. Some of medicinal plants from Indonesia have hemostasis-related activities e.g Lantana camara L., Eclipta prostrata (L.) L. dan Cosmos caudatus Kunth. Based on phytochemical screening, ethanol extract from the leaves of Lantana camara L. contains flavonoid, saponin, and essential oil, whilst the Cosmos caudatus Kunth. Leaves contained alkaloid, flavonoid, saponin, steroid, phenolic, and terpenoid.2,3 Phytochemical screening of Eclipta prostrata (L.) L.
ethanolic extract leaves showed the presence of flavonoid, quinone, tannin, steroid and triterpenoid. Previous research on other plants revealed the plants containing flavonoid, saponin and essential oil had platelet aggregation activity.

According to Middleton et al (2000), flavonoid compound can inhibit platelet aggregation by blocking the synthesis of arachidonic acid by cyclooxygenase which results the inhibition of thromboxane A2 leading to inhibition of platelet aggregation. Grice et al (2010) proved that the content of essential oils like eugenol and elemycin had antiplatelet activity as they can inhibit adenosine diphosphate (ADP) induction during platelet aggregation process. Meanwhile, Lee et al (2005) demonstrated that antiplatelet activity of saponin can inhibit the action of calcium ion as during platelet aggregation process calcium ion can promote vasoconstriction. On the grounds of the aforementioned findings, the presence of the three compounds may inhibit the formation of platelet aggregation.

One of studies on Eclipta prostrata (L.) L., was to evaluate the anti-inflammatory using carragenin to generate strong anti-inflammatory effect on rats. The research on Lantana camara L. and Cosmos caudatus Kunth. as platelet antiaggregation agents has not been found yet. However, a study revealed that Lantana camara and Cosmos caudatus had anti-inflammatory activity and the result from this study can be explored further to investigate their platelet anti-aggregation activities as the inflammatory process is associated with the formation of thrombus in the blood vessel.

**MATERIALS AND METHODS**

It was an experimental study to examine the potential of antiplatelet aggregation in reducing plasma absorbance from ethanol 70% extracts from the leaves of Lantana camara L., Eclipta prostrata (L.) L. and Cosmos caudatus Kunth. Consequently, it may prove the potential activity of the extracts to inhibit platelet aggregation.

**Materials**

Dry leaf of Lantana camara L., Eclipta prostrata (L.) L. and Cosmos caudatus Kunth. (obtained from Balitro, Bogor), ethanol, Adenosin Diphosphate (ADP) (Sigma-Aldrich), Platelet Rich Plasma (PRP) (Indonesian Red Cross, DKI Jakarta), clopidogrel (Kimia Farma), NaCl 0.9% (Otsuka), amyl alcohol, chloroform, ammonia, anhydrous acetic acid, sulfuric acid, NaOH, ferric (III) chloride, gelatine, Steasny reagent, Sodium acetate, Dragenthoff reagent, Mayer reagent, petroleum ether, and aquadest.

Testing was initiated by determination of the plants by Herbarium Bogoriense, Botany Department of Biology Research Centre (LIPI), Bogor. Extraction was done by mazeration method with 70% ethanol to obtain concentrated extract followed by phytochemical screening.

There were three treatments of the test group: positive control, negative control and extracts from each test plant. The extracts were made into a series of concentrations (40; 60; 80; and 100 mg/ml). Each 2.5 g of extracts was weighed and then dissolved into 25 ml of aquadest and homogenized to obtain 100 mg/ml concentration. Dilution of the concentration of 100 mg/ml was carried out into 10 ml of aquadest to obtain concentration 80 mg/ml, then diluted again to get 60 mg/ml concentration, and diluted again to get concentration 40 mg/ml.

Samples of test extracts (40; 60; 80 and 100 mg/ml), clopidogrel 2 mg/ml (positive control) and aquadest (negative control) were pipetted with 100 μl, then added to 450 μL platelet preparation (PRP) on a closed tube. Each tube was homogenized with vortex for ±3 min. The procedures were replicated 3 times for each sample. The absorbance test of the sample was measured at 600 nm using Spectrophotometer visible (UV Mini 124 Shimadzu). Before measuring the absorbance, 20 μL of ADP 5 mM was added to each sample. Then, the samples were incubated for ±20 min, then plasma absorbance of the samples were measured again using the same wavelength. Finally, percent inhibitory percentage for each extract was calculated according to the following formula:

\[
\text{Percentage of platelet aggregation} = \frac{1 - \text{B}}{\text{A}} \times 100\%
\]

Denote:  
\[
\begin{align*}
\text{B} &= \text{absorbance after ADP addition} \\
\text{A} &= \text{absorbance before ADP addition}
\end{align*}
\]

\[
\text{% inhibitory of platelet aggregation} = \frac{(\text{% Aggregation of negative control} - \text{% Aggregation of negative control})}{(\text{% Aggregation of negative control})} \times 100
\]

The anti-aggregation activity of each extract was expressed as IC_{50} values (the concentration of the compound causing 50% inhibitory effects). The IC_{50} values were estimated by non linear curve-fitting and presented as their respective 95% confidence limits. One-way analysis of variance (ANOVA) followed by Tukey’s post test was used to assess the significant differences (p< 0.05) between the responses caused by the extracts. All the statistical analyses were accomplished using the computer software GraphPad Prism 3.02 for Windows (GraphPad Software, USA).

**RESULTS AND DISCUSSION**

It can be seen in Table 1 that the phytochemical screening indicated the presence of secondary metabolite compounds contained in 70% ethanol extracts from the leaves of Lantana camara L., Eclipta prostrata (L.) L. and Cosmos caudatus Kunth. The secondary metabolite compounds included flavonoid, saponin, essential oil, tannin and steroid. The compounds were believed to have activity as anti-platelet aggregation.

Based on data shown in Figure 1 and 2, testing of Lantana camara extract with concentration of 100 mg/ml resulted in the lowest aggregation percentage of 2.83% ± 2.16 with a percentage of the highest aggregation inhibition of 91.17%. The highest percentage of platelet aggregation was shown by concentration of 40 mg/ml equal to 20.15% ± 2.77 giving inhibition with value 37.15%. Compared with the measurement data of Eclipta prostrata leaf extract at concentration of 100 mg/ml resulted in the lowest percent aggregation of 8.16% ± 0.57 with the highest inhibition percentage of 74.55%, whereas at concentration of 40 mg/ml yielded the highest percentage of platelet aggregation of 19.53% ± 0.57 which gave an inhibitory value of 39.08%. Meanwhile, measurement of Cosmos caudatus leaf extract at concentration of 100 mg/ml gave the biggest percentage of inhibition (i.e 59.17%) of all concentrations (80, 60 and 40 mg/ml) with aggregation percentage 13.09% ± 0.74, while concentration of 40 mg/ml had platelet aggregation percentage of 27.63% ± 0.57 which resulted in 13.82% of inhibition percentage.

Based on observations and measurement data on platelet aggregation, the higher concentration of extracts resulted in smaller resulting aggregation value so that the lower concentration of extract used may yield the greater ability of platelets to aggregate. While the capability of platelet aggregation inhibition in this study showed that the higher concentration of extract used, yielded greater inhibitory value and the lower concentration of inhibition resulted in smaller aggregation. This difference can be seen in Figure I and Figure II.

Platelet or thrombosis is one of type of blood cells. Platelets have a small size, i.e. one-third of the size of red blood cells. Each millimeter cubic of the blood contains 300,000 platelets and life span are only 8-10 days old. Platelets are derived from megakaryocytes located in the bone marrow and have no nuclei. Platelets play an important role in the blood clotting.
Table 1: Phytochemical Screening of Ethanolic Leaf Extracts from Lantana camara L., Eclipta prostrata (L.) L. and Cosmos caudatus Kunth.

<table>
<thead>
<tr>
<th>Group of Compounds</th>
<th>Lantana camara L. leaf</th>
<th>Eclipta prostrata (L.) L. leaf</th>
<th>Cosmos caudatus Kunth. leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Essential Oil</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Quinone</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroid/ triterpenoid</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Coumarin</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2: Effect of ethanolic extract of Lantana camara L., Eclipta prostrata (L. L.) and Cosmos caudatus Kunth. leaves on in-vitro ADP-induced platelet aggregation.

<table>
<thead>
<tr>
<th>Samples</th>
<th>IC50 (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lantana camara L.</td>
<td>47.09</td>
</tr>
<tr>
<td>Eclipta prostrata (L.) L.</td>
<td>59.02</td>
</tr>
<tr>
<td>Cosmos caudatus Kunth.</td>
<td>87.26</td>
</tr>
<tr>
<td>Positive control (Clopidogrel)</td>
<td>1.12</td>
</tr>
</tbody>
</table>

Figure 1: Percentage of platelet aggregation of each extract.

Figure 2: Inhibitory percentage of platelet aggregation of each extract.
In this study, clopidogrel 75 mg with concentration of 2 mg / ml is used as a positive control at a dose of 75 mg. Clopidogrel is a drug that is very similar to ticlodipine which works by inhibiting platelet aggregation induced by ADP. This drug appears to be more rare cause thrombocytopenia and leukopenia than ticlodipine. The dose is generally 75 mg / day with or without a loading dose of 300 mg.14 Clopidogrel is a safer drug that has been widely replaced by ticlodipin. Most of the previous clinical studies have examined clopidogrel in combination with aspirin. As in the study Antiplatelet Effects of Clopidogrel Compared with Aspirin after Myocardial Infarction: Enhanced Inhibitory Effects of Combination Therapy conclude that in a prospective clinical study of platelets ex vivo, clopidogrel was more effective than aspirin in inhibiting aggregation and ADP-induced platelet activation. Clopidogrel in combination with aspirin showed a synergistic inhibitory effect after stimulating collagen and thrombin with monotherapy.19

In this study, inhibition of platelet aggregation in vitro showed the presence of anti-platelet aggregation activity of the three types of extracts. These activities are believed due to the presence of flavonoid, saponin, essential oil, tannin and steroid. Antiplatelet aggregation activity of flavonoid blocks the formation of arachidonic acid metabolism by cyclooxygenase (5). In the platelet, cyclooxygenase product is mainly thromboxane A2 which inhibits the adenylate cyclase enzyme which causes a change in ATP into cAMP, so that lower cAMP levels. CAMP serves to control the concentration of free calcium ions in platelets that cause aggregation and adhesion. Low levels of cAMP would lead to the concentration of free calcium ions into high and result in the adhesion and aggregation of platelets.20 While the content of essential oils such as eugenol or elemicin can inhibit ADP-induced platelet aggregation. Eugenol also tend to have potential in the treatment of other diseases associated with platelet aggregation, such as thrombosis, transient ischemic, inflammatory, tumor growth and increased atherosclerosis,9 and the antiplatelet action of saponins can inhibit the action of calcium because calcium ions work as a promoter vasoconstriction in blood vessel.1

The platelet aggregation inhibitory assay is performed by calculating the percent aggregation of plasma absorbance before and after addition of ADP. Aggregation value of negative control is greater than that in the group of test extract and positive control. The result occurs due to the presence of antiplatelet activity of saponins can inhibit the action of calcium ions because calcium ions work as a promoter vasoconstriction in blood vessel. In this study, inhibition of platelet aggregation in vitro showed an increased potential as an inhibitor of platelet aggregation. Of all test extracts, Lantana camara L. leaf extract has the best potency for inhibiting platelet aggregation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUMMARY

The ethanol 70% extract from leaves of Lantana camara L., Eclipta prostrata (L.) L. and Cosmos caudatus Kunth. have potential as platelet aggregation inhibitory. Increased concentrations of each plant extract showed an increased potential as an inhibitor of platelet aggregation. Of all test extracts, Lantana camara L. leaf extract has the best potency for inhibiting platelet aggregation.

REFERENCES

SUMMARY
The ethanol 70% extract from leaves of *Lantana camara* L., *Eclipta prostrata* (L.) L. and *Cosmos caudatus* Kunth. have potential as platelet aggregation inhibitory. Increased concentrations of each plant extract showed an increased potential as an inhibitor of platelet aggregation. Of all test extracts, *Lantana camara* L. leaf extract has the best potency for inhibiting platelet aggregation.