ANGIOTENSIN I-CONVERTING ENZYME INHIBITORY ACTIVITY, TOTAL PHENOLIC AND FLAVONOID CONTENT OF EXTRACT AND FRACTION OF JAM FRUIT LEAVES (MUNTINGIA CALABURA L.)

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Received: 20 April 2017, Revised and Accepted: 13 July 2017

ABSTRACT

Objective: Hypertension is one of the most common chronic diseases. Inhibitory activity of angiotensin I-converting enzyme (ACE) is effective on giving hypotensive effect. Jamfruit leaf (Muntingia calabura L.) was reported to have an excellent hypotensive effect. This research was aimed to test in the manner of in vitro the inhibitory activity of ACE.

Methods: This research used ACE kit-WST, total phenolic content, and total flavonoid from jam fruit leaf ethanol extract, hexane, ethyl acetate, and butanol fraction.

Results: The result showed that Jamfruit leaf extract had ACE inhibitory activity and the most active fraction was ethyl acetate fraction. Inhibitory concentration 50% value of the most active fraction, ethyl acetate fraction was 0.63 µg/mL. Ethyl acetate fraction also provides most flavonoid and phenolic content with a value of 10.91 mg/g extract quercetin equivalent and 74.90 mg/g extract gallic acid equivalent.

Conclusions: Ethyl acetate fraction of jam fruit leaf had most flavonoid, phenolic compound, and ACE inhibitory activity.

Keywords: Muntingia calabura, Angiotensin I-converting enzyme inhibition, Antihypertensive, Phenolic, Flavonoid.

INTRODUCTION

High blood pressure or hypertension is the most important risk factor of death and disability in the world in 2010 [1]. The prevalence of hypertension in Indonesia according to data from the Health Ministry in 2013 reached 25.8% with the highest prevalence in the Bangka Belitung by 30.9% [2]. Thus, there is a challenge in continuing to seek alternative treatment of hypertension which effective and inexpensive.

Indonesia has a variety of plants that grow well and have been used as medicine for generations. One of the most available and has many benefits the jam fruit (Muntingia calabura L.). M. calabura has been used as traditional medicine for hypertension from time to time. Research in Taiwan found their strong hypotensive effect through activation of nitric oxide signaling pathway of cherry or jam fruit leaf extract [3]. There was no more research on the hypotensive activity by jam fruit leaf extract. More research was necessary included the search for another hypotensive activity pathway. ACE inhibitors are the first-line medicine in the treatment of hypertension because of their effectiveness in decreasing blood pressure. ACE plays a role in the activation of angiotensin I to angiotensin II, which acts as a potent vasoconstrictor and also stop the vasodilatory effects of bradykinin [4]. Clinical analysis and meta-analysis showed a reduction in the morbidity of cardiovascular and death on the use of ACE inhibitors and suggest the use of ACE inhibitors as treatment of the first choice whileARB working specifically inhibit the receptor that binds to angiotensin II, given to patients who are sensitive to ACE inhibitors [5]. It is also important to classify the active chemical constituents in jam fruit leaf. The search was done through this experiment by comparing the flavonoid, phenolic content, and ACE inhibitory activity of jam fruit fractions.

METHODS

This study was conducted in Phytochemical Laboratory and Quantitative Analysis of Pharmaceutical Chemistry of the Universitas Indonesia, Depok. Work procedures done were material preparations, extractions, fractionations, ACE inhibition percentage measurements, and inhibitory concentration 50% (IC₅₀) test from the extract and also total phenolic and flavonoid content measurements on jam fruit (M. calabura L.) fractions.

Material preparation

Plant determination was conducted to confirm that we used the right plant, such as jam fruit (M. calabura L.). Plant identification result showed that sample was in Muntingiaceae Family, M. calabura L. species.

Extraction

Dry and clean powdered plant was grinded to obtain a smaller size. Extraction was done by maceration. 500 g of powdered leaves were put into maceration container. Ethanol was added to the container (until 3-5 cm above the surface). Extraction was done 2 x 24 hrs; extracts were collected and evaporated using a vacuum rotary evaporator at a temperature of 55°C with a speed of 50 rpm.

Fractionation

Fractionation was done by solvent-solvent fractionation to separate the group of compounds according to their polarity using solvents which do not mix. Fractionation was done to 50.11 g of extract using n-hexane (nonpolar), ethyl acetate (semi polar), and butanol (polar) as solvents. Extracts were put into 600:600 mL polar solvent and water. Fractions were done to obtain filtrate which is nearly colorless.

ACE Inhibition assay

ACE inhibition assay was performed using ACE kit-WST from Dojindo. Borate buffer pH 8.3 containing 380 mM NaCl was used as a buffer. Absorbance measurements carried out at a wavelength of 450 nm uses filter-based microplate reader. Samples were diluted into 6 concentrations which was 100, 25, 12.5, 6.25, 3.125, and 1.563 µg/mL. Captopril was used as a control standard.
Total phenolic content (TPC)

Determination of TPC of the sample was done using the Folin–Ciocalteu assay and followed the methods of work of Al-Saeedi and Hossain (2015) with some modifications [6]. TPC expressed as the total gallic acid equivalent (GAE). 200 mL and put in a tube, 200 mL of sample was put into the reaction tube. 1.5 mL of Folin–Ciocalteu reagent was added to the tube. Then, the tube was incubated in the dark at room temperature for 5 minutes. After 5 minutes, 1.5 mL of Na₂CO₃ 5% was added to the tube and incubated back during the time of incubation in the dark and at room temperature. After incubation measured the solution using a UV-Vis spectrophotometer at a wavelength of optimum.

Total flavonoid (TF)

TF content was determined by the method of Chang et al. [7]. A total of 0.5 mL sample was added to 1.5 mL of methanol then followed by the addition of 0.1 mL ofAlCl₃ 10% 0.1 mL of 1 M sodium acetate, and 2.8 mL of aquadest. After incubation in maximum incubation time, the absorbance was measured at the maximum wavelength. Level of TF was expressed in mg quercetin equivalent (QE)/g extract.

RESULTS AND DISCUSSIONS

ACE inhibitor activity assay

ACE kit-WST was selected for testing fast, accurate, and specific. The test was done using a microplate reader, so it was time saving and requires only small amounts reagents. Most of the conventional methods based on the principle of formation of hippuryl-histidyl-leucine (HHL) by the action of ACE. The product or hippuric acid will be read with a spectrophotometer at a wavelength of 228 nm. ACE activity readings could be disturbed by HHL which were not hydrolyzed which also resulted in strong fluorescence. By using a microplate reader, the absorbance was measured at the maximum wavelength. Level of TF was expressed in mg quercetin equivalent (QE)/g extract.

Table 1: Inhibition percentage of ACE by ethanol extract of jam fruit leaf

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Inhibition (%)</th>
<th>Regression equation</th>
<th>IC₅₀ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.37</td>
<td>65.66±2.37</td>
<td>Y=0.9755X+0.7058</td>
<td>1.25</td>
</tr>
<tr>
<td>4.18</td>
<td>58.08±1.67</td>
<td>R²=0.9958X+0.3038</td>
<td></td>
</tr>
<tr>
<td>2.09</td>
<td>52.66±1.12</td>
<td>R²=0.9755</td>
<td></td>
</tr>
<tr>
<td>1.05</td>
<td>48.27±0.83</td>
<td>R²=0.9958X+0.3038</td>
<td></td>
</tr>
<tr>
<td>0.52</td>
<td>44.60±2.34</td>
<td>R²=0.9755</td>
<td></td>
</tr>
</tbody>
</table>

ACE: Angiotensin I-converting enzyme, IC₅₀: Inhibitory concentration 50%

Table 2: Inhibition percentage of ACE by ethyl acetate fraction of jam fruit leaf

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Inhibition (%)</th>
<th>Regression equation</th>
<th>IC₅₀ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.33</td>
<td>81.24±0.13</td>
<td>Y=4.0459X+47.469</td>
<td>0.63</td>
</tr>
<tr>
<td>4.17</td>
<td>63.32±0.14</td>
<td>R²=0.9894</td>
<td></td>
</tr>
<tr>
<td>2.08</td>
<td>57.80±1.13</td>
<td>R²=0.9894</td>
<td></td>
</tr>
<tr>
<td>1.04</td>
<td>52.20±0.56</td>
<td>R²=0.9894</td>
<td></td>
</tr>
<tr>
<td>0.52</td>
<td>48.11±0.86</td>
<td>R²=0.9894</td>
<td></td>
</tr>
</tbody>
</table>

ACE: Angiotensin I-converting enzyme, IC₅₀: Inhibitory concentration 50%

Table 3: TPC content of jam fruit fractions

<table>
<thead>
<tr>
<th>Sample</th>
<th>TPC content (mg/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane fraction</td>
<td>33.61±0.62</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>74.90±1.32</td>
</tr>
<tr>
<td>Butanol fraction</td>
<td>41.94±0.83</td>
</tr>
</tbody>
</table>

TF: Total phenolic content

Table 4: Flavonoid content of jam fruit fractions

<table>
<thead>
<tr>
<th>Sample</th>
<th>TF (mg/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane fraction</td>
<td>5.53±0.42</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>10.91±0.50</td>
</tr>
<tr>
<td>Butanol fraction</td>
<td>5.63±0.83</td>
</tr>
</tbody>
</table>

TF: Total flavonoid

TF assay

Measurement of TFs was performed according to the method of Chang et al. which used aluminum chloride reagent [7]. The principle of measurement of TFs is complex formation ketone group and a hydroxyl group C ring flavonoids or the hydroxyl group at the ortho position A or B ring flavonoids with aluminum chloride [5]. The complex will provide a barbichromic shift in the absorption of flavonoids that can be tested the levels of TFs. The test result showed the value of TF of jam fruit leaves extract was 47.79 mg QE/g extract. The test was done as well to the fractions, and ethyl acetate gave the highest value of TF.

The study in Taiwan obtained results that water fraction of jam fruit leaves contain flavonoids, phenolic compounds, saponins with flavonoids, and steroids as most commonly found in the leaves of jam fruit. ACE inhibitory activity by flavonoids had been extensively tested and resulted that some of the flavonoids effectively inhibit the activity of ACE. Some flavonoids were found to provide ACE inhibitory activity present in jam fruit leaf extract is chalcone, quercetin, and genistein [14, 15]. In jam fruit leaf, ethyl acetate which gave the highest value of inhibition activity has the highest value of phenolic content and TF. This may indicates that flavonoids and other phenolic compounds in jam fruit leaves gave ACE inhibitory activity to jam fruit leaves. Further
research can be done to explore further information about active chemical constituents of jam fruit leaf as a hypotensive agent.

CONCLUSION

Ethyl acetate fraction of jam fruit leaf had the highest level of flavonoid, phenolic compound, and ACE inhibitory activity. IC$_{50}$ value of the most active fraction, ethyl acetate fraction was 0.63 µg/mL. The flavonoid and phenolic content of ethyl acetate fraction of jam fruit leaf were 10.91 mg/g extract QE and 74.90 mg/g extract GAE.

ACKNOWLEDGMENT

This study was supported by the Directorate of Research and Community Services, University of Indonesia.

REFERENCES


