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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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 1.091 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 hypertensive 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 [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 × 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 Folín–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 6% 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 of AlCl₃ 10% or 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 TFP 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 absorption at a wavelength of 228 nm [11]. ACE is an enzyme that works in a non-specific cut two amino acids from amino acid sequence of the substrate. ACE kit-WST used 3-hidrosibutirilglisil-glisil-glycine (3HB-GGG) as a substrate solution.

Previously many researches have been done using substrates glycine-glycine and gave good results [9]. Assay using ACE Kit-WST followed the principle of the assay by Lam et al. [10]. In principle, as the ACE enzyme dipeptidilkarboksi peptidase works by cutting two peptides from peptide chains on its C terminal. ACE inhibitory activity test is performed by measuring the amount of 3HB obtained from cutting two peptide substrates 3HB-GGG as a substrate solution.

Results showed that the extract had activity as an ACE inhibitor. Extract of M. calabura leaves gave IC₅₀ values of 1.25 (μg/mL) (Table 1). Inhibitory activities of ACE by ethanol extract of leaves of jam fruit were included active depended on a category by Elbi and Wagner [11]. For the fractions, IC₅₀ of ACE tests were done to fraction which had the most active ACE inhibitory activity.

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

| Concentration (μg/mL) | Inhibition (%) | Regression equation | IC₅₀ (μg/mL) |
|-----------------------|---------------|---------------------|-------------|
| 8.37                  | 65.66±2.37    | Y=0.9958x-0.3038    | 1.25        |
| 4.18                  | 58.08±1.67    | R²=0.9755           |             |
| 2.09                  | 52.66±1.12    | R²=0.9755           |             |
| 1.05                  | 48.27±0.83    | R²=0.9755           |             |
| 0.52                  | 44.60±2.34    | R²=0.9755           |             |

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

ACE kit-WST was selected for testing fast, accurate, and specific. The test was done as well to the fractions, and ethyl acetate gave the value of TF of jam fruit leaves extract was 47.79 mg/100 g. In jam fruit leaf, ethyl acetate which gave the highest activity present in jam fruit leaf extract is chalcone, quercetin, and steroids as most commonly found in the leaves of jam fruit. ACE inhibitory activity by flavonoids has 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 genisten [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 flavonoid and other phenolic compounds in jam fruit leaves give ACE inhibitory activity to jam fruit leaves.
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. IC₅₀ 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.

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