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In Vitro Angiotensin-Converting Enzyme (ACE) Inhibition Test on Extract Dayak Onion Herb (*Eleutherine americana* (Aubl.) Merr. ex K. Heyne)

Fauzia Noprima Okta, Desi Nadya Aulena*, Pramudita Ika Yuliana, Risma Marisi Tambunan

Department of Pharmacognosy, Faculty of Pharmacy, Pancasila University, South Jakarta, – 12630, Indonesia.

Corresponding Author: *desi.nadya@univpancasila.ac.id (Desi Nadya Aulena)*

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Abstract: One of the world's silent killer diseases is hypertension. Hypertension occurs when angiotensin I is converted to angiotensin II, causing vasoconstriction, which decreases sodium and water excretion. The mechanism of angiotensin I conversion to angiotensin II appears in the presence of the Angiotensin-Converting Enzyme (ACE). Flavonoids have the potential as ACE inhibitors due to their structure. Dayak onion is widely used as an antihypertensive in traditional medicine. This study aims to identify secondary metabolites, determine total flavonoid content, determine extract quality parameters, and ACE inhibitory activity of the Dayak onion herb. Dayak onion herb was extracted with 70% ethanol by kinetic maceration, and then phytochemical screening was carried out, extract quality parameters tested, and ACE inhibitory activity. The results of the examination of the quality parameters, total ash content of 4.49%, water-soluble ash content of 4.00%, acid-insoluble ash content of 0.41%, drying shrinkage of 9.70%, the water content of 5.72%, solvent residue of 0.67%, Pb heavy metal content of 0.2908 mg/kg, Cd heavy metal content of 0.0880 mg/kg, Total plate number $\leq 10^3$ colonies/g, and yeast mold numbers $\leq 10^3$ colonies/g. Dayak onions herbs extract contains flavonoid, alkaloid, saponin, tannin, triterpenoid, quinone, steroid and essential oil compounds. The total flavonoid content was 2.24%. The IC_{50} of ACE inhibitory activity was 98.5 ± 0.77 ppm. The result of this study indicates that the Dayak onion herb can be used as an alternative for antihypertensive treatment.

1. Introduction

The prevalence of hypertension as one of the silent killer diseases in the world is 9.4 million patient deaths per year (1,2). Hypertension is when the blood pressure is over 140/90 mmHg, one of the diseases that cause degenerative diseases (2). Hypertension occurs when angiotensin I is converted to angiotensin II. Angiotensin II causes vasoconstriction, which has the effect of decreasing sodium and water excretion. The mechanism of changing angiotensin I to angiotensin II occurs in the presence of the Angiotensin-Converting Enzyme (ACE). By inhibiting ACE, it can lower blood pressure (3). Hypertension can occur in all gender and all ages but increases in old age, over 50 years old (4). Less physical activity can increase the risk of hypertension in old age and relate to the thickness of the muscle heart walls, blood vessels, and hormones (5). Smoking

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habits, consuming caffeine, and physical activities are the factors that increase the risk of hypertension. (6) and environments like heavy metals and a family history of hypertension (7). Many people lack information about the risk factors of hypertension (8). So, lifestyle changes can reduce the risk of hypertension, and it is called non-pharmacological treatment. Besides non-pharmacological treatment, there is pharmacological treatment with medical therapy (9). One antihypertensive drug widely used and recommended by many guidelines is angiotensin-converting enzyme (ACE) inhibitors. ACE inhibitors (ACEI) is used as first-choice therapy for reducing blood pressure but have side effects (10). Therefore, in this study the researchers tested ACEI from Dayak onions herbs to overcome the problem of drug side effects.

Dayak onions, especially bulbs, have been used for hypertension since ancient times (5). Giving Dayak onion tea to patients with hypertension in Labuhan Batu Utara Regency, Indonesia, can reduce blood pressure. The experiment was designed only in one group. The patient took Dayak onion tea, and their blood pressure was monitored before and after. The result was a decrease in blood pressure after the patient drank Dayak onion tea. The effectiveness of Dayak onion as an antihypertensive in this study was strengthened by the statistical value of $P<0.05$ (5). According to Yuliandra (2018), ethanol extract from Dayak onion bulbs can reduce rat blood pressure with a 100 mg/kg dose and Captopril 30 mg/kg (11).

Flavonoids, the secondary metabolite compounds almost found in higher plants, have the potential as ACE inhibitors (12). Flavonoids are divided into several groups based on differences in their structure and have their respective mechanisms of ACE inhibition. Flavonoids with ortho-hydroxy in their structure can form complexes with metal ions and reduce them, assumed to have a strong affinity, and can inhibit ACE (13). Cyanidin-3-O-β-glucoside isolated from *Rosa damascena* has ACEI activity with an IC_{50} value is 138.8 µM (13). Shi et al. (2018) determine the total flavonoid content in Dayak onion flowers, bulbs, and leaves. The total flavonoid content in Dayak onion flowers is higher than in Dayak onion bulbs and Dayak onion leaves (14). Based on the literature review, there has been no experiment of antihypertensive activity using Dayak onion herbs and activity test of inhibition ACE. This study aimed to determine the total flavonoid content, phytochemical screening, and Angiotensin Converting Enzyme inhibitors (ACEI) activity of 70% Dayak onion herb or whole plant ethanol extract from Dayak onion with in vitro method.

2. Experimental Section

Dayak onion herbs were collected from Tenggarong, West Borneo. Based on taxonomic determination in Bogoriense Herbarium at Botany Department, The Center of Biology Research, LIPI, Cibinong, West Java, Indonesia, the plants used were Dayak onion (*Eleutherine americana* (Aubl.) Merr. ex K.Heyne.). Dayak onion herb washed, dried, and ground to powder. Then the dried powder of Dayak onion herb will be macerated to obtain a thick extract which will then be used to test total flavonoid content, determine quality parameters, and test for angiotensin-converting enzyme inhibition.

2.1 Extraction

A 500 g of Dayak onion herb was extracted by kinetic maceration with 5 L of 70% ethanol as the solvent. The filtrate was evaporated with a rotary evaporator at 40-45°C, rotation of 70 rpm, and vacuum pressure of 175 mmHg to get the thick extract. The yield of the extract was determined by this formula below:

$$\text{Yield} = \frac{\text{weight of extract}}{\text{weight of simplicia}} \; x \; 100\% \qquad Eq.\ 1$$

The secondary metabolites of the dried powder and the extract were determined by conducting a phytochemical screening test. (15).

2.2 Total flavonoid content

Total flavonoid content was determined using the method by Vitalini et al., with slight modification (16). Extract of Dayak onion herb (10 mL) was prepared as the test solution and added to a volumetric flask of 25 mL, then added 1 mL $AlCl_3$ and ad acetate glacial 5% v/v in methanol. The amount of 10 mg Quercetin was diluted with 10.0 mL methanol P (stock solution). Pipette 1.0 mL of stock solution and add 10.0 mL methanol into a volumetric flask. Dilution was done twice to get the concentration of quercetin 10 ppm as the standard solution. The absorbance of the test and standard solution were measured using a UV-Vis spectrophotometer at wavelength 429 nm.

2.3 Determination of quality parameters

The quality parameters measured included organoleptic, total ash content, solvent residue, water and metal content determination, and microbial contamination tests. Those parameters must state that the extract is safe to use (17). Quality parameters are divided into specific and non-specific parameters.

Heavy metal contamination test

Atomic Absorption Spectrophotometer was used to determine the levels of Pb and Cd (16). The test solution was prepared from the residue of acid-insoluble total ash content. The residue was diluted with 5 mL of 10% Nitric acid and filtered. 10 mL of aqua dest was added. An amount of 1.0 mL of standard Cd and Pb with the concentration of 1000 µg/mL was diluted with aqua dest to 100 mL, thus prepared as the standard solution. A 5.0 mL of 10% nitric acid was diluted with aqua dest to 10 mL as a blank solution. The absorbance of test, standard, and blank solutions were measured using Atomic Absorption Spectrophotometer (18).

Microbial contamination test

The Total Plate Number and Yeast Mold Numbers were carried out to determine the microbial contamination test (18). Total Plate Number (TPN) and Yeast Mold Numbers (YMN) were carried out using 1 g of Dayak onion herb extract added with phosphate buffer pH 7.2 to 10.0 mL, the concentration of this solution was 10^{-1} g/mL. Make dilutions up to 10^{-6} g/mL. Pipette 1.0 mL of each dilution into a Petri dish, and pour 15-20 mL of Nutrient Agar (NA) medium (45 ± 1°C) for TPN and Potato Dextrose Agar (PDA) for YMN. The Petri dishes were incubated at 35-37°C for 24-48 hours and 20-25°C for 3-5 days for TPN and YMN, respectively.

2.4 Angiotensin Converting Enzyme Inhibition Test

Preparation of phosphate buffer with sodium chloride

A 177.4614 g of potassium dihydrogen monophosphate, 17.55 grams of sodium chloride, and 48.16 grams of sodium hydroxide were dissolved in aqua dest up to 1000.0 mL (300mM, pH 8,3).

Preparation of control solution

The amount of 25 mg captopril was diluted in 100.0 mL buffer phosphate solution pH 8.3 with sodium chloride 300 mM. Captopril solution was made in 10, 12, 14, 16, 18, and 20 ppm concentrations.

Preparation of test solution

The amount of 25 mg Dayak onion herb extract diluted in phosphate buffer pH 8.3 with sodium chloride 300 mM ad to 100.0 mL. The test solution was made in different concentrations of 100, 120, 140, 160, 180, and 200 ppm.

Preparation of ACE solution

The amount of 0.25 U/mg ACE powder was diluted into 1.0 mL buffer phosphate pH 8.3 with sodium chloride 300 mM, then take 80 µL enzyme solution and diluted with phosphate buffer solution pH 8.3 with sodium chloride 300 mM ad to 5 mL. The concentration of enzyme solution is 4 mU/mL.

Preparation of the substrate

A 21.5 mg hippuryl-L-histidine-L-leucine (HHL) was diluted into 10.0 mL buffer phosphate pH 8.3 with sodium chloride 300 mM into the volumetric flask (the final level of HHL was 5mM).

Determination of the maximum wavelength of hippuric acid

The amount of 20 mg of hippuric acid was diluted into 100.0 mL of aqua dest. 2.0 mL of the solutions were taken and then added into a 100.0 mL volumetric flask, diluted with aqua dest. The absorbance was measured using a UV-Vis spectrophotometer at a 400-200 nm wavelength.

Preparation of hippuric acid as a standard solution

A 20 mg of hippuric acid was diluted into 100.0 mL aqua dest. Several volumes of the standard solution were taken and then added to a 10.0 mL volumetric flask ad with aqua dest to make the concentration of 2, 4, 6, 8, and 10 ppm. The absorbance was measured at a wavelength of 228 nm using a UV-Vis spectrophotometer.

ACE inhibitory activity test

1. Blank solution

A 50.0 µL buffer phosphate pH 8.3, 50.0 µL substrate solution, and 50.0 µL ACE solution were added into the tube reaction, then incubated for 30 minutes at 37°C. Then 200.0 µL HCl 1 M was added, extracted with 1.5 mL ethyl acetate, and centrifuged for 15 minutes at 4000 rpm. 1,0 ml supernatant was taken into a vial and evaporated at room temperature for 2 hours. After the final solution was dried, add 3.0 mL aqua dest and measure the absorbance at a wavelength of 228 nm using a UV-Vis spectrophotometer.

2. Positive control solution

Captopril as positive control solution was prepared in different concentrations (10,12, 14, 16, 18, and 20 ppm) in buffer phosphate solution pH 8.3 with sodium chloride 300 mM in a total volume of 5.0 mL. Captopril solution (50.0 mL), a substrate solution (50.0 mL), and ACE solution (50.0 mL) were put into a tube reaction, then incubated for 30 minutes at a temperature of 37°C. Then, 200.0 mL HCl1 M was added, extracted with 1.5 mL ethyl acetate, and centrifugated for 15 minutes at 4000 rpm. A 1.0 mL supernatant was added into a vial and evaporated at room temperature for 2 hours. After the final solution was dried, add 3.0 mL of aqua dest and measure the absorbance at a wavelength of 228 nm using a UV-Vis spectrophotometer.

3. Test solution

The test solution was prepared in different concentrations, 100, 120, 140, 160, 180, and 200 ppm. Captopril solution (50.0 mL), a substrate solution (50.0 mL), and ACE solution (50.0 mL) were added into a tube reaction, then incubated for 30 minutes at 37°C. Add 200.0 µL of HCl 1 M and extract the solution with 1.5 mL ethyl acetate, then centrifuge for 15 minutes at 4000 rpm. A 1.0 ml supernatant was taken into a vial and evaporated at room temperature for 2 hours. After the final solution was dried, add 3.0 mL aqua dest and measure the absorbance at a wavelength of 228 nm using a UV-Vis spectrophotometer.

The absorption obtained was converted into a curve of standard solution (hippuric acid) to calculate the concentration of hippuric acid. The hippuric acid concentration was used to calculate the percentage of inhibition (% inhibition). Then the % inhibition obtained was used to determine the IC_{50} value.

$$\% \text{ inhibition} = \frac{\text{the concentration of hippuric acid in the blank (ppm)} - \text{concentration of test solution (ppm)}}{\text{the concentration of hippuric acid in the blank (ppm)}} \text{x} 100\% \quad \textit{Eq.}\ 3$$

3. Result

3.1 Extraction and phytochemical screening

In this section, we elaborate on the extract yield and phytochemical screening results of Dayak onion herb. Figure 1 shows a picture of the Dayak onion herb that was used.

Figure 1. Dayak onion herb (*Eleutherine americana* (Aubl.) Merr. ex K. Heyne).

Dayak onion herbs were sliced and dried at < 55 ºC. The dried Dayak onion herb bulb was ground to powder and then extracted by kinetic maceration with 70% ethanol as the solvent. This method was chosen based on its principle. Extraction is carried out at room temperature with kinetic energy to break up the plant cells. The solvent used is 70% ethanol. Ethanol is more efficient in cell wall degradation, so more secondary metabolites will be extracted. The extract yield from this method was 10.78%.

Table 1. Result of phytochemical screening of extract and dried powder of Dayak onion herbs.

Group	Dayak onion herb		
	Dried powder	**Extract**	**Color**
Alkaloid	+	+	Red precipitate (Dragendroff)
	+	+	White precipitate (Mayer)
Flavonoid	+	+	Yellow in amyl alcohol layer
Saponin	+	+	Foam
Tannin	+	+	White precipitate (gelatin)
Quinone	+	+	Red
Steroid	+	+	Green
Triterpenoid	+	+	Red-purple
Essential Oil	+	+	An Aromatic Odor
Coumarin	-	-	-

Description: (+) = detected, (-) = not detected

The secondary metabolites in dried powder and extract of Dayak onion herb were identified by phytochemical screening. Secondary metabolites include alkaloids, flavonoids, saponin, tannin, quinone,

steroids, triterpenoids, essential oil, and coumarin (19). Phytochemical screening of extract and dried powder of Dayak onion herb were presented in Table 1.

3.2 Total flavonoid content

The total flavonoid content of the Dayak onion was 2.24 %. The percentage of total flavonoid content is obtained using the formula below:

$$\% \text{ TPC } = \frac{Concentration\ of\ quercetine\ (Abs\ sample\ with\ AlCl3 - Abs\ sample)}{(Abs\ Quercetin\ with\ AlCl3 - Abs\ Quercetin)} x\ 1{,}25\ x\ \frac{100}{weight\ of\ sample}\ Eq.\ 2$$

3.3 Determination of quality parameters

Specific parameters

Organoleptic analysis is important in the evaluation of plant extract quality as it provides sensory information about the extract's appearance, aroma, flavor, and texture (20). These characteristics can be indicative of the extract's composition and purity, and can help identify any potential contamination or adulteration. In addition, organoleptic analysis can also provide insight into the extract's potential acceptability and consumer appeal, which is important for the development of new natural products (21). Table 2 shows the organoleptic analysis results of Dayak onion herb.

Table 2. The results of organoleptic analysis of the 70% ethanol extract of Dayak onion herbs.

Organoleptic	Result
Form	Thick
Color	Red
Smell	Aromatic
Flavor	Specific taste

The organoleptic test was determined using the five senses to indicate the characteristics of the extract, like form, color, smell, and flavor. The 70% ethanol extract of Dayak onion herb was a thick red extract with an aromatic odor and a specific taste.

Non-specific parameter

Non-specific parameter analysis of plant extracts is important to obtain a broad understanding of the extract's physical and chemical properties such as the presence of inorganic substances, moisture content, and purity. The total ash content and acid-insoluble total ash content determine the extract's mineral content, while the water-soluble ash content indicates the extract's quality. Residue of solvent and dry shrinkage analysis are used to assess the purity of the extract, while water content measurement is important for quality control purposes. These non-specific parameter analyses are essential in determining the quality and safety of plant extracts (22). Table 3 shows the results of non-specific parameter analysis of Dayak onion herbs.

Table 3. The results of non-specific parameters of 70% ethanol extract of Dayak onion herbs.

Parameters	Result (%)
Total ash content	4.49
Acid-insoluble total ash content	0.41
Water soluble ash content	4.00
Residue of solvent	0.67
Dry Shrinkage	9.70
Water content	5.72

The result of the non-specific parameter is presented in Table 3. The results meet the requirements. Water content is less than 10%. This result meets the requirement of BPOM, the National Agency of Drug and Food Control in Indonesia, to prevent mold growth during storage (17). The microbial test was carried out to ensure the extract's absence of bacteria and fungi.

Heavy metal contamination

Heavy metal contamination in plant extracts is a major concern due to its potential adverse effects on human health. Plant extracts may become contaminated with heavy metals from various sources such as the soil, water, and industrial pollutants. Heavy metal toxicity can lead to various health problems such as respiratory, gastrointestinal, and neurological disorders (23). Therefore, the analysis of heavy metal contamination in plant extracts is essential to ensure their safety for human consumption. Table 4 shows the heavy metal contamination levels of Dayak onion herbs.

Table 4. The results of Heavy metal contamination of 70% ethanol extract of Dayak onion herbs.

Metals	Result (mg/kg)	Requirements (mg/kg)
Pb	0.2908	≤ 10
Cd	0.0880	≤ 0.3

Table 4 indicated that the Pb contamination was 0.2908 mg/kg. This follows the required requirement of ≤ 10 mg/kg (24). The result of Cd contamination was 0.0880 mg/Kg. This also follows the required requirement of ≤ 0,3 mg/Kg (24). This result indicated the extract was safe to use. The level of heavy metal contamination is obtained using the formula below:

$$Concentration\ of\ test\ solution = \frac{Abs\ of\ test\ solution}{Abs\ of\ standard}\ x\ Concentration\ of\ standard \qquad Eq.\ 3$$

$$\text{Level of heavy metal contamination} = \frac{Concentration\ of\ test\ solution}{Weight}\ x\ Dilution\ factor \qquad Eq.\ 4$$

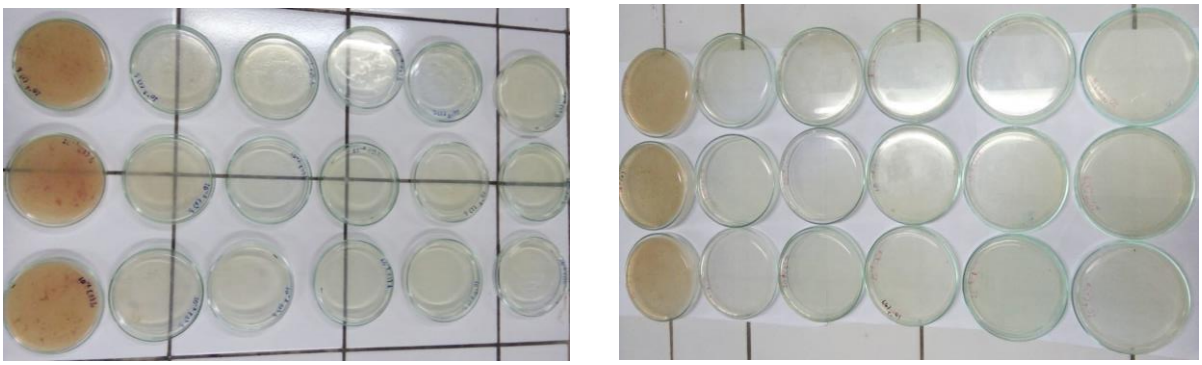
Microbial contamination

Microbial contamination in plant extracts is a potential health hazard due to the presence of pathogenic microorganisms such as bacteria, fungi, and viruses. Plant extracts can become contaminated during the harvesting, storage, or processing stages, leading to an increased risk of infections and diseases. Therefore, microbial contamination analysis is essential to ensure the safety and efficacy of plant extracts. Table 5 shows the total microbial contamination levels of Dayak onion herbs.

Table 5. The results of Microba contaminant of 70% ethanol extract of Dayak onion herbs.

Metals	Result (mg/kg)	Requirements (mg/kg)
Total Plate Number	≤ 10^3	≤ 10^4
Yeast Mold Number	≤ 10^3	≤ 10^4

The result indicated that the 70% ethanol extract of Dayak onion herbs had too few yeast mold numbers to be counted because the colonies in each growth medium were ≤ 10^3 colonies/g. This value is below the maximum limit of microbial contamination based on the Monograph of Indonesian Medicinal Plant Extracts, namely the total plate number and yeast mold numbers of ≤ 10^4 colonies/g (17). Then the resulting extract meets the quality requirements of the extract. Figure 2 shows the process of microbial contamination analysis of Dayak onion herb extract.



(a) (b)

Figure 2. Microbial contamination test: (a). Total Plate Number; (b). Yeast Mold Number.

3.4 Angiotensin Converting Enzyme Inhibition Test

Preparation of hippuric acid standard curve

Hippuric acid was the compound that resulted from a reaction between the substrate, Hipuryl-Histidine-Leucine (HHL), with ACE. Hippuric acid was the parameter in testing the antihypertensive activity of the Angiotensin Converting Enzyme (ACE) inhibitor. Determination of the maximum wavelength of hippuric acid to determine the most optimum absorption between 400-200 nm. From the absorption, hippuric acid provides the most optimum absorption at a wavelength of 228 nm. The standard curve of hippuric acid was used to calculate the concentration of the test and control solutions. Captopril was used as a control (antihypertensive drug of the ACE inhibitor class), while 70% ethanol extract of Dayak onion herb was used as a test solution. The standard curve of hippuric acid can be seen in Figure 3.

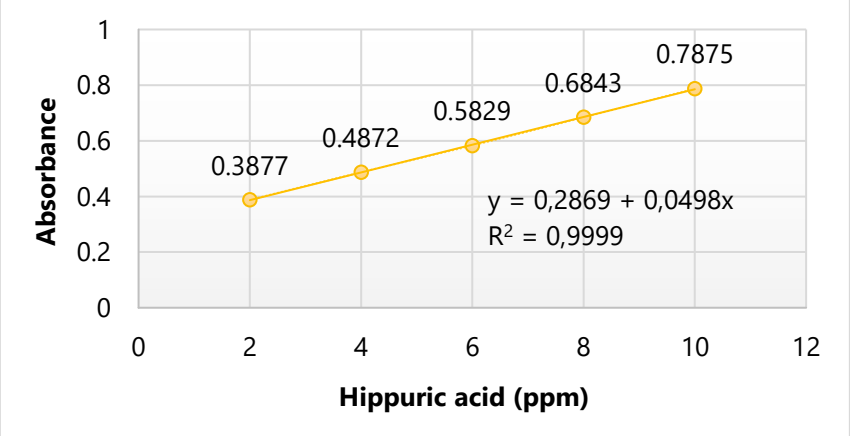


Figure 3. Standard curve of hippuric acid concentration and absorbance.

Angiotensin converting enzyme inhibition test

ACE inhibitory tests were used on a blank solution (without adding extract), captopril as a positive control, and test solution (70% ethanol extract of Dayak onion herb). Captopril and the extract are used to inhibit the complex of hippuric acid from the breakdown reaction between HHL with ACE. The absorbance of hippuric acid was measured using a UV-Vis spectrophotometer at a maximum wavelength of 228 nm. The absorbance of captopril and extract was converted to the equation of hippuric acid standard curve (see Figure 3) y = 0.2869 + 0.0498x to get concentrations of hippuric acid in blank, captopril, and test solution. The absorbance of the blank was 0.5062, and the concentration of hippuric acid in the blank was 4.4036.

$$\%\ \text{inhibition} = \frac{\text{the concentration of hippuric acid in the blank (ppm)} - \text{concentration of test solution(ppm)}}{\text{the concentration of hippuric acid in the blank (ppm)}} \text{x} 100\ \% \quad Eq\ 5$$

The correlation of captopril concentration and test solution was used to calculate the percentage of inhibition to get the IC_{50} value by this formula:

$$IC50 = \frac{50 - a}{b}, \qquad y = a + bx \quad Eq.6$$

The Percentage inhibition of captopril was obtained using the formula in Equation 5. The concentration of hippuric acid in captopril was used as the concentration of the test solution. The Percentage inhibition of 70% ethanol extract of Dayak onion herb was obtained using the formula in Equation 5. The concentration of hippuric acid in the extract was used as the concentration of the test solution, with the concentration of hippuric acid in the blank being 4.4036 ppm. The standard curve of captopril and ethanol extract of Dayak onion herbs can be seen in Figure 4.

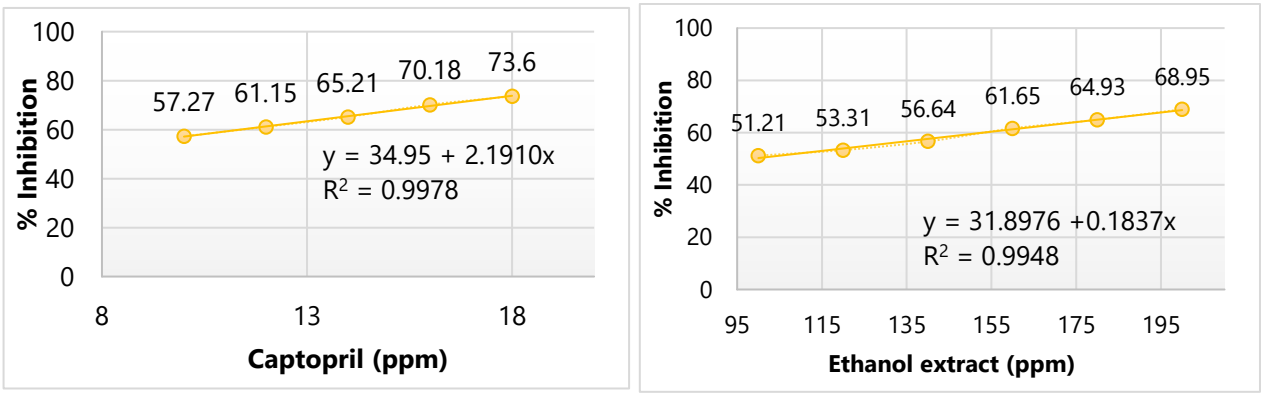


Figure 4. Standard curve of captopril and 70% ethanol extract of Dayak onion herb.

The IC_{50} value was calculated to determine the concentration of captopril and the test solution that can inhibit 50% of ACE. The IC_{50} value of captopril was obtained by using the formula (Equation 6) with using regression linear (y= 34.95 +2.1910x) (see Figure 4). The data showed that the captopril used as a positive control had an IC_{50} of 6.9 ± 0.16 ppm. The IC_{50} value of the extract was obtained using the formula (Equation 6) with linear regression (y = 31.8976 +0.1837x). The test solution has an IC_{50} of 98.5 ± 0.77 ppm.

4. Discussion

Many factors can cause hypertension, making hypertension one of the causes of death worldwide and a disease that initiates heart disease. Dayak onion herb, with its secondary metabolite content, has the potential as an antihypertensive drug, as evidenced by this research on the inhibition of ACE.

In this study, one of the target compounds is a flavonoid. The solvent used is 70% ethanol. Flavonoids are polar compounds that can be extracted using polar solvents, like 70% ethanol. Besides, 70% ethanol is a universal solvent. Universal solvent is a solvent that can extract most of the polar compounds and a small portion of semi-polar compounds and non-polar compounds. Four extracts of *Syzygium polyanthum* leave (aqueous, methanol, ethyl acetate, and n-hexane) were tested for their ability to inhibit ACE. Water extract had the highest activity against ACE, followed by hexane, methanol, and ethyl acetate extracts. Ethyl acetate has the lowest inhibitory activity (25). Secondary metabolite compounds have been known to play an active role as antihypertensive agents, such as flavonoids, alkaloids, and carotenoids (26).

Flavonoids are potential antihypertensive agents because their structure flavonoids have C2-C3 double bond, ACE inhibitory activity, and can be used to manage hypertension disorder in pregnant women (27,28). The content of polyphenols can reduce the risk of hypertension (29–31). Flavonoids, polyphenols, and

alkaloids are found in the Dayak onion herb, and the total flavonoid content of 70% ethanol extract of Dayak onion herb was 2.24%.

This research indicates that 70% ethanol extract of Dayak onion herb has the potential as an antihypertensive agent to inhibit ACE. ACE inhibitory activity was carried out using the Cushman and Cheung procedure (32). In this method, an enzyme was used as one of the main components. A substrate is also needed that will bind to the ACE enzyme. The substrate used was Hipuryl-Histidine-Leucine (HHL) because HHL has a similar amino acid structure to the peptide end of angiotensin I, which is a natural substrate in the human body (33,34). Based on previous research, flavonoids are known to inhibit the action of ACE through their ability to chelate metal ions such as zinc (12), but not all activity of ACEI through chelation with zinc. Aqueous extract of *S. polanthum* has inhibitory activity against ACE through protein precipitation (25).

Apple skin extract has a high content of flavonoids and exhibits inhibitory activity against the ACE, with an IC_{50} value was 49 ppm (12). ACE Inhibitory activity of *Veronica biloba* and *Schoenoplectus triqueter* was found at IC_{50} values of 210.68 and 229.40 ppm, respectively (27). 70% ethanol extract of Dayak onion herb has a stronger ACE inhibitory activity than these *V. biloba* and *S. triqueter* extracts.

One of the conditions for using extracts is that the extract must meet both specific and non-specific quality parameters and other tests to ensure the extract is safe to be consumed. Specific quality parameters are to ensure the identity of the extract. Non-specific quality parameters, heavy metal contamination, and microbial contamination test to ensure the extract does not contain harmful foreign materials. Total ash content indicates the presence of inorganic compounds after irradiation, which comes from the plant itself or foreign material originating from sand and soil attached to plants. To prove the safe use of the extract, besides testing the ash content, heavy metal contamination was also carried out to ensure the foreign material in the extract was harmless. Microbial contamination testing is carried out to ensure that the extract must not contain pathogenic microbes and non-pathogenic microbes exceeding the specified limits because it will affect the stability of the extract and be harmful to health.

To overcome the distinct aroma and flavor of Dayak onion, its extract can be formulated into instant granule form or other encapsulation systems (35–37). Nanotechnology can also play a significant role in enhancing its bioavailability and delivering Dayak onion components in a targeted and controlled release manner, thereby increasing its anti-hypertensive effectiveness with a long therapy duration (37,38). Additionally, more studies could be conducted to investigate the safety and efficacy of these delivery systems in pre and clinical settings.

5. Conclusion

Dayak onion herb is known to have ACE inhibitory activity with IC_{50} of 98.5 ± 0.77 ppm. The 70% ethanol extract that was tested has met the quality standard parameters required for both specific and non-specific parameters. ACE inhibition shown by 70% ethanol extract of Dayak onion herb makes Dayak onion a potential antihypertensive agent. There have been previous studies examining the antihypertensive activity of Dayak onion bulbs. To determine the effectiveness of Dayak onion bulbs and herbs as antihypertensives, it is necessary to conduct tests simultaneously to compare the effectiveness of those.

Data Availability

The data is available upon request to the corresponding author.

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Conflict of Interest

The authors declare no conflicting interest.

Authors contribution

Conceptualization : Risma Marisi Tambunan
Investigation : Pramudita Ika Yuliana
Supervision : Desi Nadya Aulena
Administration : Desi Nadya Aulena, Fauzia Noprima Okta
Writing and Editing : Fauzia Noprima Okta

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