Standardization of Indonesian Traditional Antihypertensive Medicines (Jamu) through the ACE Inhibitor Mechanism

by Aprilita Rina Yanti Eff
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ABSTRACT
Introduction: Herbal medicine (jamu) is a traditional Indonesian drug that has been used by the community in efforts to overcome health problems. One of the herbs that are frequently used by the public is antihypertensive jamu. This study aimed to determine the standardization parameters of 8 antihypertensive jamu in the form of specific and nonspecific parameters, antioxidant and angiotensin-converting enzyme inhibitor (ACEi) activity. Materials and methods: Jamu were extracted using ethanol. Nonspecific parameters that are water content, ash content, ash insoluble acid content, level of substances dissolved in alcohol and water, Coliform microbial contamination, and mold/yeast numbers. Determination of specific parameters including determining organoleptic (color and texture), chemical content, identification of infrared spectrum, in-vitro antioxidant activity, and ACEi inhibitor activity. Results: Nonspecific parameter such is the average water content of 5.92-8.1% w/w, total ash content of 5.85-7.2% w/w, levels of ash insoluble acid content were 0.45-0.55%, and the level of substances dissolved in alcohol and water were 24.22-54.21 and 24.22-64.21, respectively. The eight extracts were uncontaminated with coliform, mold, and yeast microbes. Antioxidant and ACE inhibitor activity test showed that all eight extracts had antioxidant activity in vivo with IC50 values ranging from 8.31 - 1579 ppm and ACE inhibitor activity with the IC50 value is in the range of 18.37-740.8 ppm. Conclusion: The eight antihypertensive jamu met the standard of extract parameters both specific and nonspecific and have potential in-vitro activities as ACE inhibitors. Key words: Herbal medicine (jamu); Antihypertensive; ACE inhibitor; Antioxidant.

INTRODUCTION
The use of traditional medicines have been widely known from developing to developed countries. In some developing countries, traditional medicines are used for health voices at the primary level while in developed countries the use of traditional medicines is growing rapidly. The use of traditional medicine in Indonesia has been carried out for centuries but its use is still empirical and its effectiveness and safety have not been supported by scientific data. Jamu is one of the Indonesian traditional medicines made from native sources, like roots, bark, flowers, seeds, leaves, and fruits or using ingredients obtained from animals, such as honey, royal jelly, milk and eggs. The use of herbal medicine is still done traditionally, for example in the form of steeping powder or liquid containing all ingredients of plants that make up herbs. Herbal medicine is an option for some people to maintain health because the price is inexpensive, there are no side effects on the body and the raw materials are easy to find. Prevalence of hypertension in Indonesia based on the results of measurements blood pressure by 25.8%. Most of the cases of hypertension in the community (63.2%) undiagnosed. Basic Health Research Data (Rukesdas) in 2013 showed that 33% of households in Indonesia utilize health services traditional, including 39% of houses stairs use traditional medicinal herbs. Meanwhile, Riskesdas in 2010 showed 60% of the population Indonesia was over the age of 15 years stated he once drank jamu, and 90% of them indicated their existence the benefits of drinking jamu.

Using traditional drug as part of the treatment of hypertension increasingly increased in the last decade. This matter caused by several factors, especially the prices of traditional medicines considered cheaper with less side effect. The goal of treatment of hypertension with medicinal plants is to treat high blood pressure by correcting the cause according to the philosophy of medicinal plants as constructive medicine, namely repairing/building aged organs or systems that cause hypertension. Medicinal plants have advantages in the treatment of hypertension because generally, medicinal plants have a function other than managing hypertension as well as treating comorbidities or complications as a result of high blood pressure.

One of the drugs used to restore blood pressure in patients with hypertension obtains Angiotensin Converting Enzymes (ACE) inhibitor. ACE inhibitors are the drugs of choice because, in addition to treating hypertension, they are especially beneficial for cardiovascular diseases, namely congestive heart failure and left ventricular dysfunction, enhancing function and anatomy of arteries, improving endothelial function, regressing and stabilizing atherosclerotic plaques and in diabetic nephropathy.

Cite this article: Yanti Eff AR, Rahayu ST, Mahayasih PG, Januarko MU. Standardization of Indonesian Traditional Antihypertensive Medicines (Jamu) through the ACE Inhibitor Mechanism. Pharmacog J. 2020;12(3):422-9.
Medicines included in ACE inhibitors work by inhibiting the effects of angiotensin II as a vasoconstrictor. The role of ACE inhibitors in hypertension in addition to reducing levels of angiotensin II also increases levels of bradykinin, which contributes as a vasodilator. Vasodilation decreases peripheral vessel resistance, preload, and afterload in the heart, thereby reducing blood pressure.5

Some medicinal plants are identified to produce antihypertensive effects and work by inhibiting ACE. ACE inhibitors derived from nature have safety and economic value. ACE inhibitors derived from natural product generally comes from groups of compound peptides, proanthocyanidin, terpenoids and tannins.24

The standardization of antihypertensive herbs (jumis) needs to be done to anticipate global competition in the field of jumis and the availability of antihypertensive jumis that are safe, effective, and scientifically tested. Standardization of jumis will provide a scientific foundation for the use of herbal medicine empirically through research based on health services and community welfare.2

This study aims to standardization and saniification antihypertensive jumis through inhibition of the Angiotensin-Converting Enzyme to anticipate global competition in the field of herbal medicine and the availability of safe, effective and scientifically tested antihypertensive herbs.

MATERIAL AND METHODS

Materials

The test material consisted of 8 antihypertensive herbal brands (jumis) purchased from drugstores in Jakarta, ethanol (Brachem), Aquades (Brachem), Aquamineral (Brachem), Ethyl Acetate (Merck), DMSO (Merck), Hippuric Acid (Sigma), Captopril (Sigma), HHL (Sigma), ACE from rabbit lung (Sigma), NaOH (Merck), Boric Acid (Sigma).

Methods

Extraction and Identification of the specific and non-specific parameters

The eight (8) antihypertensive herbs (jumis) were extracted with ethanol by maceration then concentrated until a thick extract was obtained. Determination of non-specific parameters includes the examination of acid insoluble ash content, water content, water-soluble extract content, ethanol-soluble compound, and microbial contamination using the ALT method. Determination of specific parameters includes organoleptic, extract chemical content and identification of infrared spectrum.

In-vitro antioxidant activity assay

Antioxidant activity assay was carried out in a test tube by measuring the absorbance DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent solution and the extract solution. A total of 1.5 ml extract was added 3 ml DPPH and mixed until homogeneous. This mixture was incubated for 30 minutes, and then the absorption was measured at a wavelength of 516 nm. Methanol is used as a blank and Vitamin C as reference drug. Free radical scavenging activity the extract is determined by calculating IC50 value, which represents the effective concentration required to reduce 50% uptake intensity compared to the reagent solution. IC50 was calculated of a percent (%) of various absorbance reductions extract concentration using linear regression.

Preliminary test of inhibition of angiotensin-converting enzyme activity

Preliminary Test Inhibition of Angiotensin-Converting Enzyme Activity included optimum determination of wavelength, temperature, incubation time, and substrate concentration. Determination of the maximum wavelength is carried out by using hypuric acid at a wavelength of 200 - 400 nm. The optimum incubation time test was taken for 15, 30, 60, 90, and 120 minutes. The optimum temperature was determined by incubating the test solution at temperatures of 30, 32, 35, and 40°C. Determination of optimum substrate concentration was performed using Hppuryl-Histidyl-Leucine (HHL) substrate at concentration of 2, 4, 5, 6, 8, and ten mM.

ACE inhibitory activity assay measurement

extracts of the antihypertensive jumis were accurately measured to inhibit the specific activity of Angiotensin-Converting Enzyme (ACE). The examination was conducted out using a spectrophotometer under aerobic conditions. The excellent results of the optimization procedure was applied to measure the ACE activity against the extract objectively accurately.

Test solution of 50 µl was gently put into a test tube, then 50 µl of HHL substrate solution was added thoughtfully to the optimum concentration. In addition, pre-incubation of the test solution was carried out at 37°C for 10 minutes. After pre-incubation, 100 µl of the ACE solution is added to the test tube and homogenized using a vortex mixer. The prepared mixture was incubated at optimum temperature and optimum time. Then immediately added 250 µl HCl 1 N to dilute the reaction. The formed hippuric acid was carefully extracted using 3 ml ethyl acetate. The prepared mixture was centrifuged for 10 minutes, and an ethyl acetate layer was gently taken at 37°C and evaporated at 100°C for 5 minutes. The formed precipitate was dissolved with 3 ml distilled water, and the absorption was precisely measured using a spectrophotometer at a maximum wavelength. Analyses with the similar procedure was carried out on Captopril as a reference drug. Boric acid buffer solution as blanks and control blanks. ACE enzyme solution was added to the blank solution mixture, while the control blanks do not include enzymes.

Percent inhibition is calculated using the formula A / B x 100%, where A = (absorbance of the blank solution) absorbance control blank – absorbance of the sample solution. B = (Absorbance of the blank solution – absorbance of the blank control solution) absorbance of control sample.

RESULTS

The composition of plants used in antihypertensive jumis can be seen in Table 1.

The results of measuring non-specific and specific parameters of each extract can be seen in Tables 2 and 3. In-vitro measuring antioxidant activity (IC50) and ACE inhibitor activity (IC50) can be seen in Table 4 and 5.

Results of preliminary test of inhibition of Angiotensin-Converting Enzyme activity show that the maximum absorption of hippuric acid is at a wavelength of 246 nm. The optimum incubation time remains 90 minutes. The optimum incubation temperature is shown at 37°C, and the optimum substrate concentration is present at eight mM.

DISCUSSION

The utilization of traditional medicines aimed at maintaining health and treating diseases in developing countries is grow rapidly. Indonesia has many medicinal plants that are potentially to be used as traditional prescriptions and have been used for generations. Traditional medicine in Indonesia is known as jumis. Some medicinal plants in Indonesia possess antihypertensive properties. National survey results show that 46.4% of Indonesian people suffer from hypertension, but only about 9% receive adequate treatment. Patients with chronic hypertension...
Table 1: Composition of plants in antihypertensive jamu.

| No | Composition of Plants                                      |
|----|------------------------------------------------------------|
| 1  | Abietis Cortex, Cuminum domesticum, Rhizoma, Zingiber rhizoma |
| 2  | Aegle marmelos, Orthosiphon sambuceus, Zingiber aromaticum Rhizoma, Abietis Cortex, Ipomoea batatas |
| 3  | Morinda citrifolia, Artocarpus integrifolia, Abietis Cortex |
| 4  | Phenanthrusrubrus, Centella asiatica, Zingiber rhizoma, Impatiens radiata, Aegle marmelos |
| 5  | Pteris cretica, Dracaena loureiroi, Zingiber rhizoma, Impatiens radiata, Aegle marmelos |
| 6  | Pteris cretica, Dracaena loureiroi, Zingiber rhizoma, Impatiens radiata, Aegle marmelos |
| 7  | Abietis Cortex, Andrographis paniculata, Cuscuta asiatica, Orthosiphon sambuceus, Phyllanthus niruri folium |

Table 2: The non-specific parameters each extract of jamu.

| No of extracts | water content (w/w) | ash content (w/w) | Ash insoluble acid content | Level of substances dissolved in alcohol (%) | Level of substances dissolved water (%) | Coliform microbial contamination (colony/g) | mold/yeast numbers (colony/g) |
|----------------|---------------------|-------------------|-----------------------------|---------------------------------------------|------------------------------------------|-------------------------------------------|-------------------------------|
| 1              | 5.6±0.45            | 6.4±0.66          | 0.1±0.02                    | 45±2±2.5                                    | 24±2±2.99                                | negative                                  | negative                      |
| 2              | 6.0±0.24            | 6.0±0.66          | 0.0±0.01                    | 58±3±1.89                                   | 35±3±1.83                                | negative                                  | negative                      |
| 3              | 8.1±0.93            | 7.2±0.36          | 0.4±0.09                    | 61±3±3.34                                   | 25±2±1.38                                | negative                                  | negative                      |
| 4              | 6.5±0.02            | 4.6±0.68          | 0.2±0.02                    | 57±3±0.35                                   | 28±3±0.68                                | negative                                  | negative                      |
| 5              | 7.6±0.03            | 4.0±0.24          | 0.1±0.05                    | 49±3±0.8                                    | 27±3±0.9                                 | negative                                  | negative                      |
| 6              | 7.2±0.58            | 4.7±0.43          | 0.2±0.02                    | 43±1±1.7                                    | 29±1±1.06                                | negative                                  | negative                      |
| 7              | 6.7±0.35            | 6.2±0.52          | 0.0±0.07                    | 53±3±1.93                                   | 29±3±1.06                                | 32±1±1.06                                | negative                      |
| 8              | 5.8±0.24            | 5.9±0.18          | 0.1±0.07                    | 66±1±1.33                                   | 31±1±1.72                                | negative                                  | negative                      |

Table 3: The specific parameters each extract of jamu.

| No of extracts | Organoleptic (colour and texture) | Chemical content | Identification of infrared spectrum |
|----------------|-----------------------------------|------------------|-------------------------------------|
| 1              | yellowish-brown and paste         | Flavonoid, alkaloid, tannin, saponin, triterpenoid | C=O, N=O, N=H, C=O, C-H (aromatic), -OH |
| 2              | yellowish-brown and liquid         | Flavonoid, alkaloid, tannin, saponin, triterpenoid | C=O, N=O, C-H (aromatic), N=H, -OH |
| 3              | yellowish-brown and paste          | Flavonoid, alkaloid, tannin, saponin, triterpenoid | C=O, N=H |
| 4              | yellowish-brown and paste          | Flavonoid, tannin, saponin, sterol | C=O, C-H (aromatic), C=O-H (aliphatic) |
| 5              | yellowish-brown and paste          | Flavonoid, tannin, saponin, sterol | C=O, C=O-H (aromatic), C=O-H (aliphatic) |
| 6              | yellowish-brown and paste          | Flavonoid, tannin, saponin, sterol | C=O, C=O-H (aromatic), C=O-H (aliphatic), C=O, alkane |
| 7              | yellowish-brown and paste          | Flavonoid, tannin, saponin, sterol | C=O, C=O-H (aromatic), C=O-H (aliphatic) |
| 8              | yellowish-brown and liquid         | Flavonoid, alkaloid, tannin, saponin, sterol, triterpenoid | OH, C=O (aliphatic), C=O, C=O (aromatic), C=N, C=H (aromatic), C=O-H (aromatic) |

Table 4: In vitro antioxidant activity (IC₅₀ (ppm)).

| No of extracts | IC₅₀ (ppm) |
|----------------|-----------|
| 1              | 93.36     |
| 2              | 79.8      |
| 3              | 78.68     |
| 4              | 74.43     |
| 5              | 11.4      |
| 6              | 67.85     |
| 7              | 357.9     |
| 8              | 11.4      |

Table 5: ACE inhibitor activity (IC₅₀ (ppm)).

| No of extracts | IC₅₀ (ppm) |
|----------------|-----------|
| 1              | 740.8     |
| 2              | 319.5     |
| 3              | 455.98    |
| 4              | 292.15    |
| 5              | 18.37     |
| 6              | 475.97    |
| 7              | 265.3     |
| 8              | 103.75    |
tend to control their blood pressure through various methods, one of which is by using antihypertensive herbs.\(^4\)\(^,\)\(^5\)\(^,\)\(^6\)

Therefore, that traditional medicines can be used in health care facilities, and the availability of safe and nutritious herbal medicine needs to be standardized. Standardization of herbal medicine refers to regulations of Indonesia’s Ministry of Health No. 1109/Menkes/Per/IX/2007 regarding the administration of effective treatment alternative, complementary health facilities. Standardization represents a critical stage in conducting research and drug development of natural medicine in Indonesia to guarantee quality and safety of these drug preparations. In this research, standardization of antihypertensive jamu extract carried out which involves non-specific parameters, for example water content, ash content, and acid insoluble ash content, and specific parameters particularly organoleptic, chemical content, and IR chromatogram pattern.\(^7\)\(^,\)\(^8\) Based on the results presented in Table 2, the water content of the herbal extract obtains 5.67-7.92% w/v or less than 10%, so that the eight extracts fulfilled standard specifications. The water content in the extract is barely than 10% minimize the growth of fungi and mold so that the durability and quality of the extract when storage remains good. Ash content measurement is intended to determine the quantity of inorganic material or minerals left after the gravimetric process. The eight herbal extracts have a total ash content of 2.68-7.24%. This value complies with the standard requirements for total ash content, which is equal to no more than 10.2%. The physical properties of an ingredient or extract can be influenced by the levels of inorganic or mineral compounds contained in the extract.

Determination of acid-insoluble ash content aims to determine the amount of ash content obtained from external factors or contamination originating from sand or soil. The Indonesian Ministry of Health requires that the acid insoluble ash level should not be more than 0.7%. The measurement results show that the eight herbal extracts meet the acid insoluble ash content standard. Determination of acid-insoluble ash content is proposed to assess extracts from earth and sand contamination. Determination of the concentration of extract in the solvent water and ethanol is an indicator of the number levels of compounds that can be found. The physical properties of plants determine the amount of compound content that can be dissolved in solutions. The results showed that the herbal extract has the highest solubility in water, which is 45.25 - 60.12%. The eighth extracts show more soluble in alcohol solvents, so when formulating the product of the extract can use alcohol solvents.\(^9\)

Testing for bacterial contamination is one of the tests to measure the purity of the extract. This test comprises the determination of the number of microorganisms allowed to indicate the absence of certain bacteria in the extract. The test results showed that the eight extracts were uncontaminated with coliform, mold, and yeast microbes. The maximum limit of microbial contamination in herbal medicines is present 1x10⁵ colonies/g while the maximum limit of yeast mold contamination is 1 x 10⁴ colonies/g.\(^9\)

Organoleptic was done by observing the physical form of the extract as an initial introduction to use the senses. Determination was done by describing the shape and color. Extracts 1, 3, 4, 5, 6, and 7 are yellow-brown in color and the form of a paste. While extracts 2 and 8 are yellow-brown and liquid. Physicochemical screening aims to find out the existence of groups of secondary metabolites contained in the extract and can also describe extract content qualitatively. Physicochemical screening results showed that the extract contained flavonoids, alkaloids, tannins, saponins, quinones and triterpenoids. Infrared Spectroscopy is a method that can observe the interaction of molecules with electromagnetic radiation that is in the wavelength region of 0.75-1000 μm or at wave numbers 13,000-10 cm⁻¹. This method can provide useful information for qualitative and quantitative analysis, as well as assist in the application of the formula for building a compound.\(^9\)

Antioxidant activity test results showed that all eight extracts had antioxidant activity in vitro with IC₅₀ values ranging from 9.31 - 157.9 ppm. As a comparison, vitamin C was used with an IC₅₀ value of 5.32 ppm. Jung et al. divided the intensity of the activity of antioxidant strength in 5 categories, very active, if the IC₅₀ value ≤ 10 ppm; active if the IC₅₀ is 10-99 ppm; moderate if the IC₅₀ value is 100-199 ppm; weak if the IC₅₀ value is 200-500 ppm and not active if the IC₅₀ value is > 500 ppm. From Table 4 it can be seen that herbal extracts number 1, 4, 7 and eight are classified as very active antioxidants, extracts numbers 2, 3 and five are classified as active antioxidants, and extract number 6 is classified as moderate antioxidants.\(^10\)

DPPH is an oxidizing agent that can be a free radical in testing antioxidant activity assay. Using this method is easy, simple, sensitive, fast, and requires a smaller sample. The test was done by calculating the value of IC₅₀, namely the concentration of the test extract that can capture 50% of free radicals obtained through the regression equation. The smaller the IC₅₀ value of a test compound, the more effective it is as a free radical antioxidant. The ability to reduce DPPH radicals in herbal extracts is related to compounds contained in it, namely compounds polyphenols and tannins. Polyphenol compounds and tannins can donate hydrogen. Antioxidant activity of these compounds occurs in the coseason of radical chain reactions that occur. Compounds that have antioxidant activity will react with DPPH through electron administration from antioxidant compounds to DPPH. This reaction causes a decrease in the color intensity of the solution Purple DPPH. The greater the concentration then the intensity of the purple color decreases DPPH that can be measured using absorption UV-VIS spectrophotometer at a wavelength 516 nm.\(^11\)\(^,\)\(^12\)\(^,\)\(^13\)

The preliminary test of ACE inhibitory activity aims to determine the optimum conditions for enzyme activity so that it can take place optimally on the following sample measurements. Optimization was done because the rate of the reaction catalyzed by enzymes is influenced by several factors, including temperature, pH, and substrate concentration.\(^14\) In the preliminary test, the maximum wavelength is determined using hippuric acid, incubation time, incubation temperature, and substrate concentration to be used at the time of testing. In the preliminary investigation, pH optimization was not carried out because based on the optimal pH incubation literature in the test was 8.3.\(^15\)\(^,\)\(^16\)\(^,\)\(^17\)\(^,\)\(^18\) The higher the absorption is obtained, the more products are produced, so the enzyme activity becomes greater. Based on the preliminary test results of ACE inhibitory activity, optimum conditions were acquired at 246 nm wavelength, the optimum incubation time of 90 minutes, substrate concentration of 8 mM, and incubation temperature of 37°C.

ACE inhibitory activity assay was performed on captopril (as reference drug) and herbal extract sample no. 1-8. The results showed that the IC₅₀ value is in the range of 265.3-740.8 ppm. Synthetic ACE inhibitors such as Captopril, Ramipril, and Lisinopril have side effects such as dry cough, hyperkalemia, rash, dizziness, and changes in taste. Therefore it is developed ACE inhibitors derived from natural materials both from food or plants. ACE inhibitors from natural ingredients are considered safer than synthetic replace ACE inhibitors. Secondary metabolites produced by plants are natural compounds that are identified as ACE inhibitors, namely flavonoids, hydrolysed tannins, xanthones, procyanidin, and caffeoylquinic acid. Several studies have shown that many ACE inhibitors come from plants but the identification that the active compounds is still minimal. In Indonesia, various plants have been used to treat hypertension. Some Indonesian medicinal plants that have antihypertensive activity, namely Feronia americana Mill, F. microcarpa (Schick) Boerl., Osaxis corniculata Linn, Catharanthus
Clinical study of the efficacy of boiled Herb compared to stepped hypertension carried out by Triyono et al. consists of celery and kaffir leaves, Ginger rhizomes, Tamarindus indica, and Phyllanthus herbes. Steeping herbal remedy exerts the effect of reducing blood pressure (systolic and diastolic) and increasing the SF-36 quality of life score equivalent to boiled Herbs. Steeping herbal medicine and herbal decoction exerts the effect of reducing blood pressure to normal (nornormetensive), each of 63% and 41% of the research subjects. Steeping herbal medicine can eliminate clinical symptoms of hypertension (dizziness/headache stuff neck/wrinky and rheumatic pain) the subject of the study is moderately faster than the herbal decoction.23

Some classes of antihypertensive drugs that are often used as monotherapy treatment are diuretics, β-blockers, Angiotensin-converting enzyme (ACE), Angiotensin receptor blocker (ARB), and Calcium channel blockers (CCB). The use of combination therapy is given if treatment with monotherapies has not been able to control blood pressure correctly. Antihypertensive drug combinations that are frequently used comprise a combination of diuretics, β-blockers, and ACE-inhibitors. Hypertension herbal medicine is also a combination of several dried powder medicinal plants that have different mechanisms of action. The combination of several medicinal plants is expected to have a synergistic effect from the chemical content of some medicinal plants in lowering blood pressure.23

Jamu extract number 5 possesses the slightest IC50 value of 18.37 ppm. This herbal extract consists precisly of several medicinal plants, namely: Plumeria macrocarpa, Gymnura procumbens, Imperata cylindrica, Centella asiatica, and Syzygium polyanthum.

Plumeria macrocarpa is one of Indonesia’s native plant1 which owns medicinal properties. Empirical leaves and fruits have been used to treat various types of illnesses like cancer, liver, heart disease, diabetes, rheumatism, kidney disease, asthma, strokes, and high blood pressure.24

Mahalakshmi Dewa contains immortu s anti-inflammatory, 5-oxo-3-cholestanol, and cholesterol, 5-oxo-3-cholestanol, and cholesterol.25

Mahalakshmi Dewa additionally exerts an antioxidant effect which works to inhibit alfa glucosidase and has an 8.36% diabetogenic effect in rats induced by streptozotocin.26-27 The results of a study conducted by Bhattacharya et al. showed that the extracts of leaves and fruits have activity as ACE inhibitors with IC50 values on leaves are 189.31 ppm in petroleum ether, 157.74 ppm in ethyl acetate and 107.50 ppm in methanol.28-29

Gymnura procumbens is used for the treatment of various diseases like hypertension, vasodilators, fever, diabetes mellitus, and hyperlipidemia in Indonesia.26 Gymnura procumbens water extract in rats has anti-hypertensive effects, decreases lactate dehydrogenase levels, creatin kinase, and increases nitric oxide. Ethanol extract of G. procumbens can reduce triglyceride and serum cholesterol levels in streptozotocin-induced diabetic rats. The antidiabetic effect of G. procumbens ethanol extract is comparable to Biguanide. G. procumbens increases glucose metabolism via the glycolysis pathway and inhibits endogenous liver glucose production through the gluconeogenesis pathway.30

Extracts of G. procumbens have activity as an ACE inhibitor with an IC50 value of 431.54 ppm in petroleum ether, 227.41 ppm in ethyl acetate and 452.69 ppm in methanol.
CONCLUSION

The eight antihypertensive jams met the standard of extract parameters both the specific and non-specific and have potential activities as ACE inhibitors.

ACKNOWLEDGEMENT

The authors express their gratitude to the Indonesia Ministry of Research, Technology and Higher Education for the funding this research.

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GRAPHICAL ABSTRACT

The 8 antihypertensive herbs (jamu) were extracted with ethanol by maceration.

- Non-specific parameter: ash content, acid insoluble ash content, water content, water-soluble extract content, ethanol-soluble compound, and microbial contamination.

- Specific parameters: arganoleptic, extract chemical content and identification of infrared spectrum.

In-vitro Antioxidant activity assay: Free radical scavenging activity the extract is determined by calculating IC50 value.

Preliminary Test of Inhibition of Angiotensin-Converting Enzyme Activity: optimum determination of wavelength, temperature, incubation time, and substrate concentration.

ACE Inhibitory Activity Assay: The examination was conducted using a spectrophotometer.

- The eight antihypertensive jamu met the standard of extract parameters both the specific and non-specific.
- Antioxidant and ACE inhibitor activity test showed that all eight extracts had antioxidant activity in vitro with IC50 values ranging from 9.31 - 157.4 ppm and ACE inhibitor activity with the IC50 value is in the range of 18.37-240.8 ppm.

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**Cite this article:** Yanti Eff AR, Rahayu ST, Mahayasih PG, Januarko MU. Standardization of Indonesian Traditional Antihypertensive Medicines (Jamu) through the ACE Inhibitor Mechanism. Pharmacog J. 2020;12(9):422-9.
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