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

by Aprilita Rina Yanti Eff

Submission date: 20-Jul-2020 06:29PM (UTC+0700)
Submission ID: 1359918273
File name: PharmacognJ-12-3-422.pdf (431.08K)
Word count: 6564
Character count: 37384

Pharmacogn J. 2020; 12(3): 422-429
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

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

Aprilita Rina Yanti Eff[1,*], Sri Teguh Rahayu[1], Putu Gita Mahayasih[1], Muhammad Unggul Januarko[2]

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 ACE inhibitor activity. **Results:** nonspecific parameter such is the average water content of 5.92-8.1 v / w; total ash content of 5.85-7.2 w / w, levels of ash insoluble acid content were 0.45-0.55 w/w and the level of substances dissolved in alcohol and water were 24.22-54.21 and 24.22-54,21, 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 vitro* with IC_{50} values ranging from 9.31 - 157.9 ppm and ACE inhibitor activity with the IC_{50} value is in the range of 18.37-740.8 ppm. **Conclusion:** The eight antihypertensive jamu met the standard of extract parameters both the specific and nonspecific and have potential *in-vitro* activities as ACE inhibitors.

Key words: Herbal medicine (jamu); Antihypertensive; ACE inhibitor; Antioxidant.

Aprilita Rina Yanti Eff[1,*], Sri Teguh Rahayu[1], Putu Gita Mahayasih[1], Muhammad Unggul Januarko[2]

[1]Department of Pharmacy Faculty of Health Science, Universitas Esa unggul, Jakarta, 11510, INDONESIA.
[2]Department of Management Faculty Economics and Bussiness, Universitas Esa Unggul Jakarta, 11510, INDONESIA.

Correspondence
Aprilita Rina Yanti Eff
Department of Pharmacy Faculty of Health Science, Universitas Esa Unggul, Jakarta, 11510, INDONESIA.
E-mail: aprilita.rinayanti@esaunggul.ac.id

History
- Submission Date: 27-12-2019;
- Review completed: 12-01-2020;
- Accepted Date: 05-02-2020

DOI : 10.5530/pj.2020.12.65

Article Available online
http://www.phcogj.com/v12/i3

INTRODUCTION

The use of traditional medicines have been widely known from developing countries to developed countries. In some developing countries, traditional medicines are used for health services 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 scientifically data.[1]

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.[2] 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 (Riskesdas) in 2013 showed that 30.4% of households in Indonesia utilize health services traditional, including 49% 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.[3]

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.[4] 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 damaged 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.[5]

One of the drugs used to restore blood pressure in patients with hypertension obtains an 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 the 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.[6]

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.[7,8]

The standardization of antihypertensive herbs (jamu) needs to be done to anticipate global competition in the field of jamu and the availability of antihypertensive jamu that are safe, efficient, and scientifically tested. Saintification of jamu will provide a scientific foundation for the use of herbal medicine empirically through research based on health services and community welfare.[9]

This study aims to standardization and saintification antihypertensive jamu 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 (Jamu) purchased from drugstores in Jakarta, ethanol (Bratachem), Aquadest (Bratachem), Aquademineral (Bratachem), Ethyl Acetate (Merck), DMSO (Merck), Hipurat 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 (jamu) were extracted with ethanol by maceration then concentrated until a thick extract was obtained. Determination of non-specific parameters includes the examination of ash content, 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 (H (2,2-diphenyl-1picrylhyrazyl) 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 516 nm. Methanol is used as a blank and Vitamin C as reference drug. Free radical scavenging activity the extract is determined by calculating IC_{50} value, which represents the effective concentration required to reduce 50% uptake intensity compared to the reagent solution. IC_{50} 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-Conversion 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 out for 15, 30, 60, 90, and 120 minutes. The optimum temperature was determined by incubating the test solution at temperatures of 30, 32, 35, 37, and 40°C. Determination of optimum substrate concentration was performed using Hyppuryl-Histidyl-Leucine (HHL) substrate at concentration of 2, 4, 5, 6, 8, and ten mM.

ACE inhibitory activity assay measurement

extracts of the antihypertensive jamu 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 optimal temperature and optimum time. Then immediately added 250 µL HCl 1 N to discontinue the reaction. The formed hippuric acid was carefully extracted using 1.5 mL ethyl acetate. The prepared mixture was centrifuged for 10 minutes, and an ethyl acetate layer was gently taken at the top 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.[10,11]

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.[12]

RESULTS

The composition of plants used in antihypertensive jamu 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 (IC_{50}) and ACE inhibitor activity (IC_{50}) 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 jamu.[1] 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 Plant
1	*Alstoniae Cortex, Curcumae domesticae Rhizoma, Zigiberis rhizoma* *...eri rhizome, Alii sativi Bulbus*
2	*Apium graveolens, Orthosiphonis Folium, Zingiberis aromaticae Rhizoma, Alstoniae Cortex, Imperatae Rhizoma*
3	*Morindae Fructus, Apii Herba, Orthosiphonis Folium, Alstoniae Cortex* *Phylanthi Herba, Centellae Herba, Blumeae Folium, Plantaginis Folium, Andrographidis Herba*
4	*Morindae fructus, Phyllanthi herba, Centella herba, Centella herba, Zingiberis rhizome, Imperata radix, Alyxiae cortex*
5	*Phaleria macrocarpa, Gynura procumbens, Imperata cylindrica, Centella asiatica, Syzygium polyanthum.*
6	*Liguistrinae lignum, Andrographidis herba, Abri folium, Leucaena Glauca* Other ingredients up to 7 g, including *Philannty herba, Orthosipon folium, Alstoniae cortex, Merremia tubera, Curcuma rhizomaae, Dioscoreae tubera rhizome, Blumneae folium, Bascilici folium, Zingiberis aromatic rhizome, and Tinosporae caulis*
7	*Alstonia cortex, Andrographidis herba, Catharanthus Roseus*
8	*Curcuma xantorrhizae, Andrographis paniculata herba, Tinospora crispa, Orthosiphon stannous folium, Phylanthus niruri folium*

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

No of extracts	water content (v/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	5.67±0.45	6.42±0.06	0.15±0.02	45.72±2.5	24.29±2.99	negative	negative
2	6.04±0.24	6.04±0.06	0.06±0.01	58.37±1.89	35.5±3.83	negative	negative
3	8.1±0.93	7.24±0.36	0.45±0.09	61.1±3.34	25.28±1.38	negative	negative
4	6.82±0.2	4.63±0.08	0.28±0.02	57.62±0.55	28.38±0.68	negative	negative
5	7.68±0.03	4.8±0.24	0.61±0.95	49.2±0.8	32.76±0.9	negative	negative
6	7.29±0.58	4.70±0.43	0.27±0.02	48.2±1.17	43.1±0.36	negative	negative
7	6.74±0.35	6.24±0.52	0.09±0.07	53.33±1.93	29.19±1.06	...tive	negative
8	5.85±0.24	5.92±0.18	0.41±0.07	60,16±1.33	31.93±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 (a...tic); -OH
2	yellowish-brown and liquid	Fl...oid, alkaloid, tannin, saponin, triterpenoid	C-O; N=O; C-H (aromatic); N-H; -OH
3	yellowish-brown and paste	Flavonoid, alkaloid, tannin, saponin, steroid, triterpenoid	C=O; N-H
4	yellowish-brown and paste	Flavonoid, tannin, saponin, steroid	C=O; -OH; C=C; C-O' C-H (aromatic); C-H (aliphatic)
5	yellowish-brown and paste	Flavonoid, alkaloid, tannin, saponin, steroid, triterpenoid	C-H (aliphatic); -OH; C=C; C-N; C-O
6	yellowish-brown and paste	...vonoid, alkaloid, tannin, saponin, steroid	-OH; C-H (aliphatic); C-O; alkuna
7	yellowish-brown and paste	Flavonoid, alkaloid, tannin, saponin, quinon, triterpenoid	-OH; C-H (aliphatic); C=O; C=C (aromatic); C-N
8	yellowish-brown and liquid	Flavonoid, alkaloid, tannin, saponin, steroid, triterpenoid	OH; C-H (aliphatic); C=C; C=O; C-N; C-H (aromatic);

Table 4: *In-vitro* antioxidant activity (IC_{50}).

No of extracts	IC_{50} (ppm)
1	9.36
2	79.8
3	78.68
4	24.43
5	11.4
6	67.85
7	157.9
8	11.4

Table 5: ACE inhibitor activity (IC_{50}).

No of extracts	IC_{50} (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.[1-3,13]

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 Minister of Health No. 1109 / Menkes / Per / IX / 2007 regarding the administration of effective treatment alternative, complementary health facilities. Standardization represent a critical stage in conducting research and drug development 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 parameter, example water content, ash content, and acid insoluble ash content, and specific parameters particularly organoleptic, chemical content, and IR chromatogram pattern.[14] Based on the results presented in Table 2, the water content of the herbal extract obtains 5.67-7.92% v/w or less than 10%, so that concluded 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 graying process. The eight herbal extracts have a total ash content of 2.46-7.24%. 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 using alcohol solvents.[15]

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 and 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 $1x10^5$ colonies / g while the maximum limit of yeast mold contamination is 1×10^{-3} colonies/g.[15]

Organoleptic was done by observing the physical form of the extract aim 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 yellowish-brown in color and the form of a paste. While extracts 2 and 8 yellowish-brown and liquid. Phytochemical screening aims to find out the existence of groups of secondary metabolites contained in the extract and can also be describe extract content qualitatively. Phytochemical screening results showed that the extract contained flavonoids, alkaloids, tannins, saponins, quinones and triterpenoids. Infrared Spectrophotometry 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 wavenumbers 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.[16]

Antioxidant activity test results showed that all eight extracts had antioxidant activity *in vitro* with IC_{50} values ranging from 9.31 - 157.9 ppm. As a comparison, vitamin C was used with an IC_{50} value of 5.32 ppm. Jung *et al.* divided the intensity of the level of antioxidant strength in 5 categories, very active, if the IC_{50} value <50 ppm; active if the IC_{50} is 50-100 ppm; while if the IC_{50} value is 101-250 ppm; weak if the IC_{50} value is 250-500 ppm and not active if the IC_{50} 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.[17]

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 small sample. The test was done by calculating the value of IC_{50}, namely the concentration of the test extract that can capture 50% of free radicals obtained through the regression equation. The smaller the IC_{50} value of a test compound, the more effective it is as a free radical antidote. 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 cessation 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.[16,18]

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.[19] 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.[20] 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 IC_{50} 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 inhibitor.[8] Secondary metabolites produced by plants are natural compounds that are identified as ACE inhibitors, namely flavonoids, hydrolyzed tannins, xanthones, procyanidin, and caffeoylquinic acid. Several studies have shown that many ACE inhibitors come from plants, but the identification of active compounds is still minimal. In Indonesia, various plants have been used to treat hypertension. Some Indonesian medicinal plants that have antihypertensive activity, namely *Persea americana* Mill, *Phalleria macrocarpa* (Scheff) Boerl], *Oxalis corniculata* Linn, *Catharanthus*

roseus (L.) G. Donn), *Dendropthoe pentandra* (L. Miq), *Swietenia mahogany* L (Jack).), *Gynura procumbens* (Lorr) Merr), *Melia azedarach* L, *Hibiscus rosa-sinensis* L, *Alstoniae Cortex*, *Curcumae xanthorrhizae*, *Zingiberis aromatic*, *Cyperi rotunda*, *Alii sativi*, *Morinda citrofolia*, *Centella asiatica*, *Orthosiphonis aristatus*, *Stachytapheta mutabilis* and *Blumea balsamifera*.[5,21]

Clinical study the efficacy of boiled of Hypertension Herbs Compared to steeped hypertension carried out by Triyono *et al.* consists of *celery* herbs, *Centella* herbs, *Orthoshipon* leaves, *Ginger rhizomes*, *Turmeric rhizomes*, and *Phyllanthus* herbs. 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. Stepping herbal medicine and herbal decoction exerts the effect of reducing blood pressure to normal (normotensive), each of 63% and 14% of the research subjects. Steeping herbal medicine can eliminate clinical symptoms of hypertension (dizziness/headache stiff neck/whiny and rheumatic pain) the subject of the study is moderately faster than the herbal decoction.[22]

Some classes of antihypertensive drugs that are often used as monotherapy treatment are diuretics, beta-blockers, Angiotensin-converting enzyme inhibitors (ACEI), 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 IC_{50} value of 18.37 ppm. This herbal extract consists precisely of several medicinal plants, namely: *Phaleria macrocarpa*, *Gynura procumbens*, *Imperata cylindrica*, *Centella asiatica*, and *Syzygium polyanthum*.

Phaleria macrocarpa is one of Indonesia's native plants 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 disorders, strokes, and high blood pressure.[24] Mahkota Dewa contains mahkotaside, mangiferin, kaempferol-3-O-d glucoside, dodecanoic acid, palmitic acid, ethyl stearic, and sucrose.[25] Mahkota Dewa additionally exerts an antidiabetic effect which works to inhibit alfa glucosidase and has an antidiabetic effect in rats induced by streptozotocin.[26,27] The results of a study conducted Eff *et al.* showed that the extracts of leaves and fruits have activity as ACE inhibitors with IC_{50} values on leaves is 189.13 ppm in petroleum ether, 157.74 ppm in ethyl acetate and 101.52 ppm in methanol while the IC_{50} values in fruit were 161.7 ppm in petroleum ether, 139.11 ppm in ethyl acetate and 122.38 ppm in methanol.[28]

Gynura procumbens is used for the treatment of various diseases like hypertension, vasodilators, fever, diabetes mellitus, and hyperlipidemia in Indonesia.[29] *G procumbens* water extract in rats has anti-hypertensive effects, decreases lactate dehydrogenase levels, creatinine phosphate kinase, and increases nitric oxide. Ethanol extract of *G procumbens* can reduce triglyceride and serum cholesterol levels in sterptozotosin-induced diabetic rats. The antidiabetic effect of *G procumbens* ethanol extract is comparable to Biguanid. *G procumbens* increases glucose metabolism *via* the glycolysis pathway and inhibits endogenous liver glucose production through the gluconeogenesis pathway.[30] Extracts of G procumbens has 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 in methanol.

Imperata cylindrica roots contain twelve phenolic compounds were isolated, such as flavonoids, simple phenols, phenolic acids, coumarins, and lignans, potassium, cylindrene, and graminone B.[31] Flavonoids possess the effect of inhibiting Angiotensin Converting Enzym. Inhibition of angiotensin production causes a reduction in aldosterone secretion resulting in natriuresis, a decrease in intravascular fluid volume, and decrease in blood pressure.[32] Potassium obtains an anti-renin an enzyme that plays a role in the production of angiotensin. Potassium is additionally an aldosterone inhibitor. Potassium can decrease membrane potential, causing relaxation of vascular smooth muscle. Relaxation of smooth muscle causes the diameter of blood vessels to widen so that blood pressure drops. Cylindrene produces inhibitory activity against the contraction of vascular smooth muscle and Graminone B is a vasodilator agent.[29] The results of a study conducted by Delima and Sari, 2018 showed that the roots of *I cylindrica* affected lowering blood pressure in healthy subjects. The mean systolic and diastolic blood pressure in subjects who were given boiled *I cylindrica roots* were significantly lower than blood pressure before being given infusion ($p < 0.01$). The decoction of the weed root decreases both systolic and diastolic blood pressure in the subject. Mean systole blood pressure after taking *I cylindrica roots* infusion (95.33 ± 5.381) mmHg, lower than before drinking infusion of (107.07 ± 5.800) mmHg. The mean diastolic pressure after drinking Imperata root infusion was (64.27 ± 3.693) mmHg, lower than before drinking Imperata root infusion of (70.00 ± 3.854) mmHg.[33]

Centella asiatica is an herbal plant of the Mackinlayaceae family. *C. asiatica* can be found in countries like Indonesia, Sri Lanka, Malaysia, Australia, Iran, Melanesia, New Guinea, and other Asian countries. *C. asiatica* has chemical content, namely triterpenoids, essential oils, flavonoids, and other components. Triterpenoids are the main component of *C. asiatica* and become marker of the quality of *C. asiatica*. Triterpenes are in the form of asiatic acid, madecassic acid, and their respective glycosides, asiaticoside, and madecassoside. Essential oils such as caryophyllene, farnesol, humulene, and bicyclogerma. Flavonoids in the kind of quercetin, routine catechin, and naringin and other components in the form of polysaccharides, polyene, alkene, fatty acids, sesquiterpenes, alkaloids, sterols, carotenoids, and tannins.[34] Asiatic acid is a triterpenoid that has antioxidant, anti-inflammatory, antihyperlipidemic, and antidiabetic activity in mice induced by Streptozotocin. A study conducted by Manesa *et al.* 2016 showed that Asiatic acid exerts an antihypertensive effect, improving vascular function through the retention of endothelial nitric oxide synthase (eNOS) and p47phox expression in rats of L-NAME hypertension. Asiatic acid also has ACE inhibitor action by reducing the activity of the angiotensin renin system and increasing vascular function by decreasing sympathetic nerve activity.[35]

Bay leaves (*Syzygium polyanthum*) possesses a variety of pharmacological activities, namely as antihypertensive, antidiabetic, antioxidant, antidiarrheal, anti-inflammatory, immunomodulatory, antibacterial, and anticancer. Active compounds which play a role in its pharmacological activities are quercetin, gallic acid, caffeic acid, and phenolic acid.[36,37] Water and methanol extract of *S. polyanthum* possess antihypertensive activity in mice, caused by terpenoid phenolic (eugenol), tannins, and flavonoids compounds. Eugenol has vasorelaxant so that it can reduce blood pressure.[38] Antihypertensive effects are thought to inhibit beta-adrenergic and cholinergic receptors through nitrite oxides production.[39] *S. polyanthum* leaves have ACE inhibitor activity at a concentration of 100 ppm, with the percentage of inhibition is 53.37%.[40] *S. polyanthum* leaves extract causes vasorelaxation and inhibits Angiotensin-Converting Enzyme (ACE), thereby reducing elevated blood pressure. Studies conducted by Ismail and Wan Ahmad show that water and methanol extracts of *S. polyanthum* cause vasorelaxation in WKY and SHR mice.[39]

CONCLUSION

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

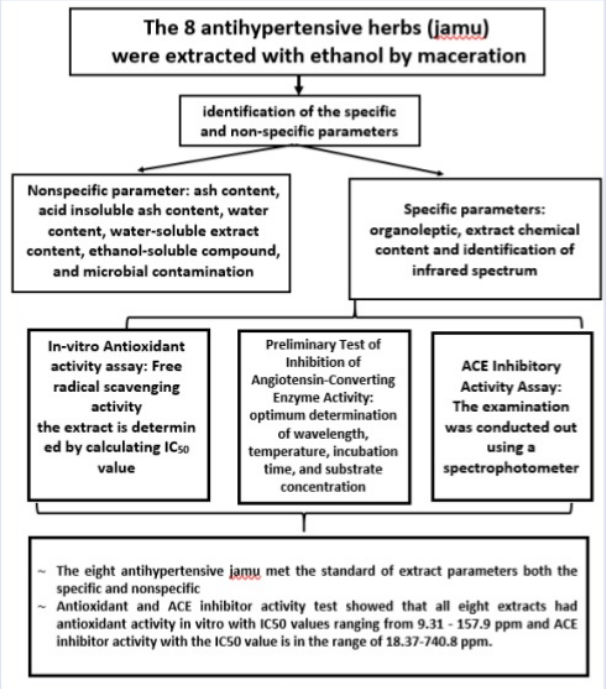
ACKNOWLEDGEMENT

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

REFERENCES

1. Herman MJ, Supardi S, Handayani RS. Policy on herbal traditional medicines therapy in three provinces in indonesia. Bul Penelit Kesehat. 2013;41(2):111-9.
2. Riswan S, Roemantio SH. Jamu as traditional medicine, in java Indonesia. South Pacific Study. 2002;23(1):1-10.
3. Indonesia M of H. Basic health research 2013. Heal Res Dev Agency, Indonesia Minist Heal. 2013;72-7.
4. Hussaana A, Sarosa H, Indrayani UD. Formula jamu antihipertensi and captopril are equally effective in patients with hypertension. Universa Med. 2016;35(2):81-8.
5. Iskandar Y. Medicinal plants that are efficacious as antihypertensive. Sci Pap. 2007;1-27.
6. Tardif J. Angiotensin-converting enzyme inhibitors and atherosclerotic plaque : a key role in the cardiovascular protection of patients with coronary artery disease. Eur Heart J. 2009;11 supplementary:E9-16.
7. Balasuriya BWN, Rupasinghe HPV. Plant flavonoids as angiotensin converting enzyme inhibitors in regulation of hypertension. Funct Foods Heal Dis. 2011;5:172-88.
8. Lettre DP, Kumar R, Kumar A, Sharma R, Baruwa A. Pharmacological review on natural ACE inhibitors. Der Pharm Lett. 2010;2(2):273-93.
9. Indonesia M of H. Regulation of the Minister of Health of Republic of Indonesia No: 003/MENKES/PER/I/ 2010 about Saintification of jamu based on research health services. 2010;pp:1-15.
10. Gao D, Chang T, Li H, Cao Y. Angiotensin I-converting enzyme inhibitor derived from cottonseed protein hydrolysate. African J Biotechnol. 2010;9(53):8977-82.
11. Meyer J, Bütikofer U, Walther B, Wechsler D, Sieber R. Hot topic: Changes in angiotensin-converting enzyme inhibition and concentrations of the tripeptides Val-Pro-Pro and Ile-Pro-Pro during ripening of different Swiss cheese varieties. J Dairy Sci. 2009;92(3):826-36.
12. Sp L, Hadira O. *In vitro* inhibitory potential of selected malaysian plants against key enzymes involved in hyperglycemia. Mal J Nutr. 2011;17(1):77-86.
13. Abascal EY, Abascal K. Treating hypertension botanically. Altern Complement Ther. 2001;October:284-90.
14. Hidayati DN, Cicih Sumiarsih UM. Non-specific standardization of leaves and caulis (*Crescentia cujete Linn*). J Ilm Cendekia Eksakta. 2005;19-23.
15. Departemen Kesehatan RI. General standard parameters of medicinal plant extracts. 2000. p. 13-30.
16. White JL. Interpretation of infrared spectra of soil minerals. Soil Sci. 2000;112(1):22-31.
17. Jun M, Fu HY, Hong J, Wan X, Yang CS, Ho CT. Comparison of antioxidant activities of isoflavones from kudzu root (*Pueraria lobata* Ohwi). J Food Sci. 2003;68(6):2117-22.
18. Garcia E, Oldoni T, Severino MA, Lugoercia A, Grande R. Antioxidant activity by DPPH assay of potential solutions to be applied on bleached Teeth. Braz Dent J. 2012;23(1):22-7.
19. Murray RK, Granner D, Rodwell V. Biokimia harper translated from harper's illustrated biochemistry 27th ed oleh Brahm U dan Nanda Wulandari. 2006.
20. Cushman DW, Cheung HS. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. Biochem Pharmacol. 1971;20(7):1637-48.
21. Indonesia pharmacists association. Daftar obat alam indonesia (DOA). III. 2008. p.55-57.
22. Triyono A, Ridha P, Ardianto D. Clinical trial the efficacy of boiled hypertension herbs compared with steeped hypertension herbs. J Ilmu Kefarmasian Indonesia. 2018;16(1):78-85.
23. Rumiyati, Arif RH, Arini DM, Dea NS. Antihypertensive testing of combination of *Apium graveolans* L., *Orthosiphon stamineus* Benth., and *Morinda citrifoliaL* extract on normotensive and hypertensive Sprague dawley Rats. Maj Obat Tradis. 2016;21(3):149-56.
24. Harmanto. Potential of the Mahkota Dewa as traditional medicine. In: One day seminar. Research and development center for pharmacy and traditional medicine of the Republic of Indonesia, Jakarta;2003.
25. Oshimi S, Zaima K, Matsuno Y, Hirasawa Y, Iizuka T, Studiawan H, et al. Studies on the constituents from the fruits of *Phaleria macrocarpa*. J Nat Med. 2008;62(2):207-10.
26. Triastuti A, Choi JW. Protective effects of ethyl acetate fraction of *Phaleria macrocarpa* (Scheff) Boerl. on oxidative stress associated with alloxan-induced diabetic rats. J Ilm Farm. 2008;5(1):9-17.
27. Sugiwati S, Setiasih S, Afifah E. Antihyperglycemic activity of the mahkota dewa [*Phaleria macrocarpa* (Scheff.) Boerl.] Leaf Extracts As An Alpha-Glucosidase Inhibitor. Makara Kesehat. 2009;13(2):74-8.
28. Rinayanti A, Radji M, Mun A, Suyatna FD. Screening Angiotensin Converting Enzyme (ACE) inhibitor activity of antihypertensive medicinal plants from indonesia. Int J Pharm Teach Pract. 2013;4(1):527-32.
29. Anwar MA, Disi SS Al, Eid AH. Anti-hypertensive herbs and their mechanisms of action: Part II. Front Pharmacol. 2016;7.
30. June CC, Wen LH, Ani HA, Latif J, Gansau JA. Hypoglycemic effect of *Gynura procumbens* fraction on streptozotocin-induced diabetic rats Involved phosphorylation of GSK3β (ser-9) in liver. Sains Malaysia. 2012;41(8):969-75
31. Liu R, Chen S, Ren G, Shao F, Huang H. Phenolic compounds from roots of *Imperata cylindrica* var. major. Chinese Herb Med. 2013;5(3):240-3.
32. Guerrero L, Castillo J, Quiñones M, Garcia-Vallvé S, Arola L, Pujadas G, et al. Inhibition of angiotensin-converting enzyme activity by flavonoids: structure-activity relationship studies. PLoS One. 2012;7(11):e49493.
33. Ellya Roa Delima YMS. The Effect of cogongrass (*Imperata cylindrica* (L.) P. Beauv) in lowering blood pressure. https://repository.maranatha.edu/12848/10/1110220_Journal.pdf.
34. Inamdar EK, Yeole RD, Ghogare AB, Souza NJ De. Determination of biologically active constituents in *Centella asiatica*. J Chromatogr A. 1996;742:127-30.
35. Maneesai P, Bunbupha S, Kukongviriyapan U, Prachaney P, Tangsucharit P. Asiatic acid attenuates renin-angiotensin system activation and improves vascular function in high-carbohydrate, high-fat diet fed rats. BMC Complement Altern Med. 2016;1-11.
36. Nor E, Abd A, Ismail A, Omar MN, Rahmat UN, Ismail A, et al. GC-MS Analysis of Phytochemical Compounds in *Syzygium polyanthum* leaves extracted using ultrasound-assisted method. Pharmacogn Journal. 2018;10(1):110-9.
37. Muhammad IR, Ester MH. Pharmacological activity, active compounds, and mechanism of action bay leaf (*Syzygium polyanthum*): Review. In: Prosiding Seminar Nasional & Workshop "Perkembangan Terkini Sains Farmasi & Klinik. 2015.
38. Estevam C, Damiani N, Venturini L, Valentim D. Vasorelaxant effects of eugenol on rat thoracic aorta. Vascul Pharmacol. 2003;40:59-66.
39. Ismail A, Mohamed M, Sulaiman SA, Ahmad WANW. Autonomic nervous system mediates the hypotensive effects of aqueous and residual methanolic extracts of *Syzygium polyanthum* (Wight) Walp. var Polyanthum leaves in anaesthetized rats. Evidence-Based Complement Altern Med. 2013;2013.
40. Muthia R, Suganda AG, Sukandar EY. Angiotensin-I converting enzyme (ACE) inhibitory activity of several indonesian medicinal plants. Res J Pharm Biol Chem Sci. 2017;8(1S):192-9.

GRAPHICAL ABSTRACT



ABOUT AUTHORS

Aprilita Rina Yanti Eff was born in Tanjung Karang, Indonesia, in 1968. He received a bachelor's degree in Pharmacy from Universitas Padjadjaran, Bandung, Indonesia, in 1992, and the Magister Biomedic and Ph.D. degrees in Medical and Pharmacy Faculty, from the Universitas Indonesia, Jakarta, in 2001 and 2010, respectively. In 2001, she joined the Department of Pharmacy, UTA 45 Jakarta University as a Lecturer. Since 2015, he has been with the Department of Pharmacy, Universitas Esa Unggul, where he has been an Assistant Professor, became an Associate Professor in 2010. Her current research interests include herbal medicine, Pharmacology, and clinical Pharmacy.

Putu Gita Maya W. Mahayasih, finished her magister program in pharmacy, at faculty of Pharmacy, Universitas Indonesia. She focused her research on natural product field. Currently, she is a lecture at Pharmacy department, faculty of health and sciences, Universitas Esa Unggul.

Moehammad Unggul Januarko, finished his magister program in Management, at faculty of Economic and Bussiness, Universitas Gajah Mada. She focused her research on management field. Currently, she is a lecture at Management department, faculty Economic and Bussiness, Universitas Esa Unggul.

Sri Teguh Rahayu, was born in Jakarta, Indonesia, in 1972. Her received a bachelor's degree in Pharmacy from Universitas 17 Agustus 1945, Jakarta, Indonesia, in 2006, she finished the Magister Pharmacy and Ph.D. degrees Pharmacy Faculty, from the Universitas Indonesia, Jakarta, in 2011 and 2018, respectively. In 2006, she joined the Department of Pharmacy, Universitas 17 Agustus 1945, Jakarta University as a Lecturer. Since 2015, he has been with the Department of Pharmacy, Universitas Esa Unggul, where he has been an Assistant Professor in 2018. Her current research interests include pharmaceutical analysis, bioanalysis and herbal medicine.

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.

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

ORIGINALITY REPORT

15%	9%	8%	9%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	ijptp.iomcworld.com Internet Source	2%
2	Submitted to Sriwijaya University Student Paper	1%
3	Submitted to Surabaya University Student Paper	1%
4	Jia P. Marcellano, Alyssa S. Collanto, Rolly G. Fuentes. "Antibacterial Activity of Endophytic Fungi Isolated from the Bark of Cinnamomum mercadoi", Pharmacognosy Journal, 2017 Publication	1%
5	Monteil-Rivera, F.. "Combination of X-ray photoelectron and solid-state ^1^3C nuclear magnetic resonance spectroscopy in the structural characterisation of humic acids", Analytica Chimica Acta, 20001201 Publication	1%
6	Submitted to Universitas Indonesia Student Paper	1%

7	Submitted to Universitas Kristen Satya Wacana Student Paper	<1%
8	Basavegowda, Nagaraj, Akber Idhayadhulla, and Yong Rok Lee. "Preparation of Au and Ag nanoparticles using Artemisia annua and their in vitro antibacterial and tyrosinase inhibitory activities", Materials Science and Engineering C, 2014. Publication	<1%
9	L Hakim. "Evaluation of five seeds of medicinal plants in the nursery level", IOP Conference Series: Earth and Environmental Science, 2020 Publication	<1%
10	Submitted to Universitas Sebelas Maret Student Paper	<1%
11	Submitted to University of the Western Cape Student Paper	<1%
12	www.longdom.org Internet Source	<1%
13	Submitted to The University of the South Pacific Student Paper	<1%
14	jifi.farmasi.univpancasila.ac.id Internet Source	<1%
15	S H Wijaya, C Fadillah, W A Kusuma. "Prediction of synergistic effect between multiple	<1%

compounds related to diabetes mellitus", IOP Conference Series: Earth and Environmental Science, 2019
Publication

16	Submitted to University of Reading Student Paper	<1%
17	R Yuniarti, S Nadia, A Alamanda, M Zubir, R A Syahputra, M Nizam. " Characterization, Phytochemical Screenings and Antioxidant Activity Test of Kratom Leaf Ethanol Extract Korth) Using DPPH Method ", Journal of Physics: Conference Series, 2020 Publication	<1%
18	journals.sagepub.com Internet Source	<1%
19	Submitted to Universitas Esa Unggul Student Paper	<1%
20	www.jyoungpharm.org Internet Source	<1%
21	Submitted to Felician College Student Paper	<1%
22	Putri Lestari, Katrin K, Rissyelly R, Putu Gita Maya Widyaswari Mahayasih. "Inhibition Activity of Angiotensin Converting Enzyme (ACE) and Determination of Total Phenolic and Flavonoid Compound from Bitter Melon Leaves	<1%

(Momordica charantia L.)", Pharmacognosy Journal, 2017
Publication

23	www.tiprpress.com Internet Source	<1%
24	Na-Ri Lee, Tae-Hun Go, Sang-Mee Lee, Seong-Yun Jeong, Geun-Tae Park, Chang-Oh Hong, Hong-Joo Son. "In vitro evaluation of new functional properties of poly-γ-glutamic acid produced by Bacillus subtilis D7", Saudi Journal of Biological Sciences, 2014 Publication	<1%
25	aip.scitation.org Internet Source	<1%
26	www.e3s-conferences.org Internet Source	<1%
27	Submitted to Indonesia Australia Language Foundation Student Paper	<1%
28	bmccomplementmedtherapies.biomedcentral.com Internet Source	<1%
29	benthamopen.com Internet Source	<1%
30	sphinxsai.com Internet Source	<1%

31	Submitted to University of Brighton Student Paper	<1%
32	medcraveonline.com Internet Source	<1%
33	jamumadura.com Internet Source	<1%
34	www.ajol.info Internet Source	<1%
35	E. Apostolidis, Y.-I. Kwon, R. Ghaedian, K. Shetty. " Fermentation of Milk and Soymilk by and Enhances Functionality for Potential Dietary Management of Hyperglycemia and Hypertension ", Food Biotechnology, 2007 Publication	<1%
36	Sheng-Lei Yan, Hui-Ting Yang, Yi-Ju Lee, Chun-Che Lin, Ming-Hui Chang, Mei-Chin Yin. "Asiatic Acid Ameliorates Hepatic Lipid Accumulation and Insulin Resistance in Mice Consuming a High-Fat Diet", Journal of Agricultural and Food Chemistry, 2014 Publication	<1%
37	R A Laeliocattleya, E Martati, A N S Alwi, L P Aulia, Yunianta. " The characteristics of corn silk (L.) herbal drinks tea with vacuum drying method as antioxidant ", IOP Conference Series: Earth and Environmental Science, 2020	<1%

	Publication	
38	Submitted to Monash University Sunway Campus Malaysia Sdn Bhd Student Paper	<1%
39	Submitted to Walailak University: The Center for Library Resources and Educational Media Student Paper	<1%

Exclude quotes	On	Exclude matches	< 10 words
Exclude bibliography	On		