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Angiotensin-I Converting Enzyme (ACE) Inhibitory Activity Of Several Indonesian Medicinal Plants

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ABSTRACT

Angiotensin-I Converting Enzyme (ACE) is known to catalyze the conversion of angiotensin I to angiotensin II, if ACE converts angiotensin I excessively, it will lead to increase blood pressure. One way to prevent the increasing in blood pressure by inhibiting ACE. The purpose of this research to determine the inhibitory activity of ACE of several medicinal plants in Indonesian and determine the plants that have the highest activity in the inhibition of ACE. ACE inhibitory activity performed by in vitro used incubation temperature conditions in 37°C, for 60 minutes and pH 8.3. Measurement of activity using spectrophotometer UV-Vis. This research used ethanol extract of several plants with a concentration 100 ppm. The results were compared with captopril as a positive control. Eighteen plants that were tested showed activity as an ACE inhibitor, wherein the ethanol extract of *Averrhoa bilimbi* L. With a concentration 100 ppm showed the highest inhibitory activity is 71,48 $\pm$ 1,71 %.This research showed that the ethanol extract of *Averrhoa bilimbi* L. showed the highest ACE inhibitory activity and deserves for further research.

Keywords: ACE, *Angiotensin-I Converting Enzyme,* Antihypertension, Ethanol Extract.

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INTRODUCTION

Hypertension is one of the most common diseases that afflict human beings in the world which is defined as high blood pressure in the artery continuously [1,2]. Hypertension is a serious public health problem throughout the world because of the high prevalence and the concomitant increase in the risk of disease [3]. Raising awareness and about the diagnosis of hypertension, to improve blood pressure control with appropriate medication, considered an important public health initiatives to reduce the morbidity and mortality of cardiovascular disease [1].

ACE is an enzyme that plays an important role in regulating blood pressure by converting angiotensin I into angiotensin II. Angiotensin II contributed to the increase in blood pressure/hypertension [2,4]. So as to prevent a rise in blood pressure is required to ACE inhibitors [5]. The use of synthetic drugs ACE inhibitors continuously give unfavorable effects to the body, so the search for alternative medicines that are safe, economical and continuously improved. The existence of other alternatives of natural products give a new hope for prevention with cheaper prices and possible side effects were smaller.

Therefore, researchers will try to explore the potential of Indonesian plant is efficacious as an antihypertensive. Antihypertensive activity test conducted by ACE inhibition method performed in vitro using UV-Vis spectrophotometry with substrateHippuryl-L-Histidyl-L-Leucine(HHL).

MATERIALS AND METHODS

Plant materials:

Fresh Plants collected from Manoko Experimental Farm, Lembang, West Java, Indonesia in May until July. There are 18 species of plants that were tested, namely herbs of *Andrographis paniculata* (Burm.f.) Ness., leaves of *Annona muricata* L., leaves of *Apium graveolens* L., leaves of *Averrhoa bilimbi* L., herbs of *Catharanthus roseus* (L.) G. Don., rhizomes of *Curcuma domestica* Val., leaves of *Cyclea barbata* Miers., leaves of *Mesona palustris* Bl., leaves of *Morinda citrifolia* L., leaves of *Morus alba* Linn., herbs of *Nasturtium officinale* R. Br., leaves of *Orthosipon stamineus* Benth., leaves of *Persea Americana* Mill., herbs of *Phyllanthus niruri* L., fruits of *Solanum indicum* Linn., fruits of *Solanum nigrum* L., leaves of *Syzigium polyanthum* (Wight) Walp., and Silk of *Zea mays*

Chemical materials:

Captopril as comparator drugs (positive control) derived from the Air Force of Pharmacy Institute,Bandung, West Java, Indonesia. ACE and HHL as a substrate for ACE obtained from PT Elo Karsa Utama, Bandung, West Java, Indonesian (Sigma Aldrich Distributor).

Methods:

Preparation Of Plant Extracts

Plant part used was collected, washed, shredded, then dried. 20 grams sample extracted using maceration method using ethanol 95 %. Extraction done for 3x24 hours . After 3x24 hours, the filtrate obtained was filtered, and then concentrated using a rotary evaporator. Condensed ethanolic extract of plants subsequently freeze dry and ready to use for the test material.

Assay for ACE Inhibitory Activity

The ACE inhibitory activity was assayed by the method Chusman and Cheung [6] with some modifications. A sample solution with concentration 100 ppm (50 µL)and 150 µL of substrate (8 mM HHL in a 100 mM sodium borate buffer containing 300 mM NaCl at pH 8.3) was pre-incubated at 37° C for 10 minutes, then added 50 µL solution of ACE (25 mU/mL), then incubated for 60 minutes at the same temperature. The reaction was stopped by the addition of 250 µLHCl 1 M. The resulting hippuric acid was extracted with 1,7 mL of ethyl acetate, and centrifuged (3000 rpm) for 15 minutes. One mL of the supernatant was transferred to another test tube and evaporated over boiling water for 45 minutes. The hippuric acid was dissolved in 3,0 mL

of deionitation water [7].The absorbance was measured at 228 nm using a spectrophotometer ultraviolet-visible (UV-Vis) Hewlett Packard 8435 [8, 9, 10, 11, 12]. Absorbance of each extract then used to calculate the ACE inhibitory activity expressed as a percentage inhibition of ACE, using the formula:

$$\text{ACE inhibitory activity } (\%) = [1 - (A-B/C-D)] \times 100$$

Where A is a solution containing the sample, substrate and ACE ; B is a solution containing the sample and ACE; C is a solution containing a substrate and ACE ; and D is a solution containing only the substrate [13,14].

Phytochemical screening.

Phytochemical screening made to the ethanol extract of plants that have the highest ACE inhibitory activity. Phytochemical screening includes qualitative examination of the class of flavonoids, quinones, saponins, phenols, tannins, alkaloids ,and steroids/triterpenoids .

RESULTS AND DISCUSSION

Results and Discussion

Extraction was done by maceration using ethanol 95 % for 3x24 hours. Maceration is an extraction method by soaking components. Maceration suitable for unknown compound its properties , as it can keep the compounds in samples that are not heat resistant to prevent damage. The yield eighteen ethanol extract can be seen in Table 1.

Table 1: The Yield Results Maceration Method

No	Species name	Part of plant	Yield (%)
1	*Andrographis paniculata* (Burm.f.) Nees.	Herbs	13,55
2	*Annona muricata* L.	Leaves	22,85
3	*Apium graveolens* L.	Leaves	22,50
4	*Averrhoa bilimbi* L.	Leaves	17,70
5	*Catharanthus roseus* (L.) G. Don.	Herbs	21,75
6	*Curcuma domestica* Val.	Rhizomes	12,50
7	*Cyclea barbata* Miers.	Leaves	12,35
8	*Mesona palustris* Bl.	Leaves	17,40
9	*Morinda citrifolia* L.	Leaves	6,10
10	*Morus alba* Linn.	Leaves	21,15
11	*Nasturtium officinale* R. Br.	Herbs	23,95
12	*Orthosiphon stamineus* Benth.	Leaves	14,10
13	*Persea Americana*Mill.	Leaves	22,80
14	*Phyllanthus niruri* L.	Herbs	27,15
15	*Solanum indicum* Linn.	Fruits	29,35
16	*Solanum nigrum* L.	Fruits	14,30
17	*Syzigium polyanthum* (Wight) Walp.	Leaves	11,15
18	*Zea mays* L.	Silk	24,41

This research used some indonesian medicinal plants that are empirically known to have the activity of antihypertensive or has been tested anthipertensi in vivo that *Andrographis paniculata* (Burm.f.) Nees., *Apium graveolens* L., *Catharanthus roseus* (L.) G. Don., *Curcuma domestica* Val., *Morinda citrifolia* L., *Nasturtium officinale* R. Br., *Orthosipon stamineus* Benth., *Solanum indicum* Linn., *Solanum nigrum* L. [4], *Zea mays* L. [15], *Annona muricata* L., *Cyclea barbata* Miers., *Syzigium polyanthum* (Wight) Walp. [16], *Averrhoa blimbi* L.[17], *Mesona palustris* Bl. [18], *Morus alba* Linn.[19], *Persea Americana* Mill. [20] and *Phyllanthus niruri* L. [21] .

In this reseacrh captopril, used as a comparator for possessing ACE inhibitory activity. Captopril is used as an agent antihypertensive therapy (Figure 1).

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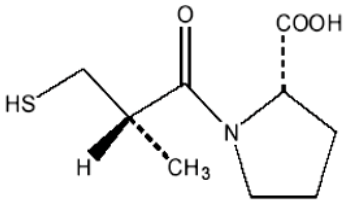

Figure 1: Structure of captopril [29]

It acts as a potent and specific inhibitor of the ACE [22], to compete with the angiotensin I, as a natural substrate, to prevent it from occurring angiotensin II . In the present research used HHL substrateas a substitute angiotensin I. HHL reacted with ACE will form histidine-leucine and hippuric acid, which hippuric acid that is formed will be measured to determine the ACE inhibitory activity [23]. Hydrolysis reaction HHL by ACE can be shown in Figure 2 [5].

At ACE inhibitory activity test, the concentration of ethanol extract of the tested plants is 100 ppm. Testing is done with captopril as a positive control. This test is performed at optimum conditions ,ie at an incubation temperature 37 ° C , pH 8.3 and incubation time for 60 min [24] . The test results were obtained in the form absorbance. The lower the absorbance values generated power greater inhibition of ACE activity. The absorbance measured residual hippuric acid derived from a reaction between the substrate and the ACE that is not inhibited by the extract of the plant. The results are summarized in Table 2.

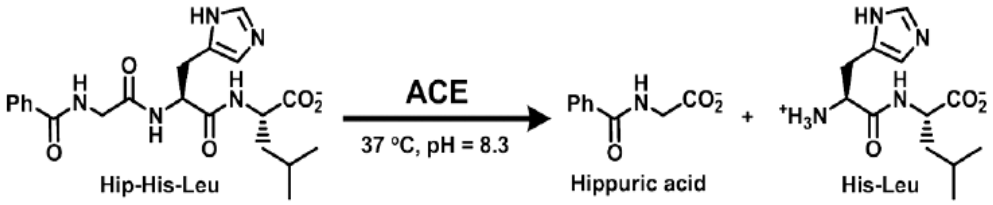

Figure 2: Hydrolysis reaction HHL by ACE

Table 2: ACE Inhibitory Activity (%) Of Ethanol Extracts From Several Indonesian Plants (Test Concentration 100 ppm)

Species name	Vern name	Family	ACE Inhibitory Activity (%)*
Andrographis paniculata (Burm.f.) Nees.	Sambiloto	Acanthaceae	29,38 ± 1,82
Annona muricata L.	Sirsak	Annonaceae	5,57 ± 2,10
Apium graveolens L.	Seledri	Apiaceae	37,91 ± 5,67
Averrhoa bilimbi L.	Belimbing Wuluh	Oxalidaceae	71,48 ± 1,71
Catharanthus roseus (L.) G. Don.	Tapak Dara	Apocynaceae	19,27 ± 5,54
Curcuma domestica Val.	Kunyit	Zingiberaceae	24,15 ± 3,21
Cyclea barbata Miers.	Cincau Hijau	Menispermaceae	35,57 ± 4,54
Mesona palustris Bl.	Cincau Hitam	Labiatae	36,25 ±5,71
Morinda citrifolia L.	Mengkudu	Rubiaceae	66,64 ± 2,32
Morus alba Linn.	Murbei	Moraceae	46,05 ± 3,07
Nasturtium officinale R. Br.	Selada Air	Brassicaceae	51,94 ± 2,92
Orthosiphon stamineus Benth.	Kumis Kucing	Lamiaceae	55,41 ± 4,03
Persea Americana Mill.	Alpukat	Lauraceae	29,49 ± 6,24
Phyllanthus niruri L.	Meniran	Euphorbiaceae	13,74 ± 1,23
Solanum indicum Linn.	Terong Ngor	Solanaceae	53,24 ± 2,88
Solanum nigrum L.	Leunca	Solanaceae	29,52 ± 5,95
Syzygium polyanthum (Wight) Walp.	Salam	Myrtaceae	53,37 ± 0,95
Zea mays L.	Jagung	Poaceae	50,44 ± 3,77
Captopril			**88,17 ± 2,89**

Mean ± Standard Deviation done in three replicate

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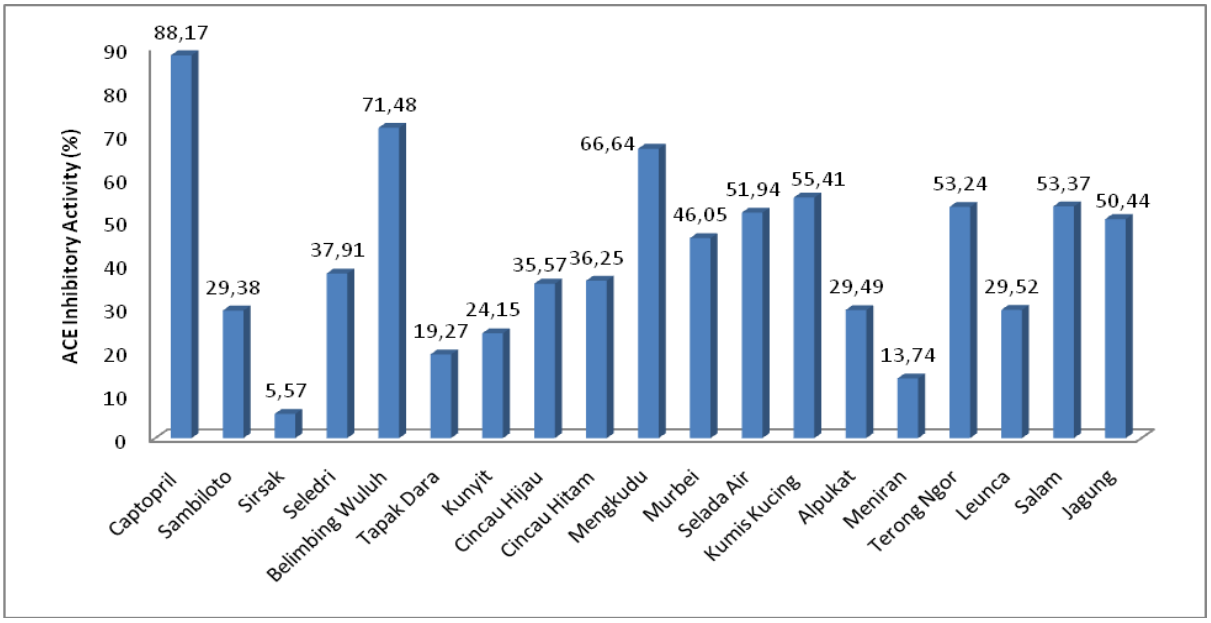

Figure 3: ACE Inhibitory Activity(%) From Plant Extracts

The test results showed that all tested extracts could potentially inhibit the activity of ACE. Ethanolic extract of *Averrhoa blimbi* L. showed the best ACE inhibitory activity (71,48 ± 1,71). Followed *Morinda citrifolia* L. (66,64 ± 2,32), *Orthosiphon stamineus* Benth. (55,41 ± 4,03), *Syzigium polyanthum* (Wight) Walp. (53,37 ± 0,95) and *Solanum indicum* Linn. (53,24± 2,88). The percentage inhibition of ACE whole extract tested is smaller compared with captopril.

Solanum indicum Linn. contain chemicals that diosgenin lanosterol, sitosterol, solasonin, solamargin, solanine, solanidin and solasidin [25]. The traditional use to treat high blood pressure, diabetes, acne and menstrual pain [26]. The results showed *Solanum indicum* Linn. has laxative activity, cardiotonic and antibacterial against pseudomonas [25, 27].

Syzigium polyanthum (Wight) Walp. contains essential oils (citral, eugenol), tannins and flavonoids [28]. Traditionally, bay leaves are used as an upset stomach [26]. The results showed regards activity antidiabetic, antibacterial, antifungal, antioxidant, antihypertensive, and antidiarrheal [29].

Leaves of *orthosiphon stamineus* Benth. have chemical content of potassium salts, ortosifon glycosides, essential oils, saponin [28] luteolin, neoortosifon, norstaminol, norstaminolakton, norstaminon, ortosifol, ortosifonon, quercetin, sifonol, staminol and staminolakton [30]. The traditional use of the leaf to diuretics, catarrh, inflammation of the kidneys, kidney stones, gallstones, arteriosclerosis, rheumatism, diabetes, high blood pressure, inflammation of the tonsils, epilepsy, menstrual disorders, gonorrhea, syphilis and albuminuria [26]. The results showed *orthosiphon stamineus* Benth. have antioxidant activity, antitumor, diuretics, antidiabetic, antihypertensive, anti-inflammatory, antibacterial and hepatoprotective. Results of acute toxicity test showed dekokta cat whiskers dose of 2 g/kg non-toxic, and extract the ethanol dose of 5 g/kg did not show any abnormalities in test animals [30].

Morinda citrifolia L. has chemical constituents of essential oils, caprylic acid, morindadiol, soranyidiol [15], scopoletin, vitamin C, nordamnakantal, morindon, rubiadin, rubiadin-1-metileter, β-sitosterol, carotene, akubin, routine [31], kaempferol, nikotiflorosida, quercetin,sitrifolinin and sitrifosida [32]. The traditional use as a high blood pressure medicine, beri- beri, launched urinary, kidney inflammation, inflammation of the bile, colitis, dysentery, constipation, pain in the spleen, the spleen swelling, liver disease, salivary bleeding, diabetes, intestinal worms, chicken pox, obesity, back pain, abdominal pain, eliminate dandruff, antiseptic, laxative menstruation and blood cleanser[26]. The results showed *Morinda citrifolia* L. has antibacterial activity, anthelmintic, an antioxidant, hepatoprotective, anti-obesity, anti-diabetic, analgesic, anti-inflammatory, antihypertensive and anti-cancer [32].

Leaves of *Averrhoa bilimbi* L. contain chemicals that dietilphtalat, felurat acid, myristic acid, phitol [33], a fruit extract contains flavonoids, saponins , triterpenoids , and citric acid [34] . The traditional use to cope with fever, bowel disease, arthritis and ulcers [26]. The results showed Leaves of *Averrhoa bilimbi* L. extract has the effect of decreasing blood pressure in test animals cats [33], antidiabetic, antihiperlipidemik, antimicrobial [34], antiinflammatory, antioxidant and antifertilitas. Results of acute toxicity test extract up to 1 g/kg for 15days did not show any toxic symptoms in test animals [35].

Based on the test results of ACE inhibitory activity against plant phytochemical screening done *Averrhoa bilimbi* L. which has the highest activity. Phytochemical screening results can be seen in Table 3.

Table 3: Phytochemical Screening Results

Compound	Ethanolic Extract
Flavonoids	+
Quinones	+
Saponins	+
Fenols & Tanins	+
Alkaloids	-
Steroids/triterpenoids	+

Results of phytochemical screening showed the compound contained flavonoids, phenols, tannins, quinones, saponins and steroids/triterpenoids. This is consistent with several studies that show flavonoid compounds, tannins hydrolyzed, xanton, and sapogenin have ACE inhibitory activity [35,36].

CONCLUSIONS

This study showed that the ethanol extract of *Averrhoa bilimbi*L. shows ACE inhibitory activity is quite large. Therefore, further research is needed to find the ACE inhibitors active compound of *Averrhoa bilimbi* L.

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