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INHIBITION OF ANGIOTENSIN CONVERTING ENZYME (ACE) ACTIVITY BY SOME INDONESIA EDIBLE PLANTS

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ABSTRACT: Antihypertensive properties of plant can be evaluated by *in vitro* method on inhibition of Angiotensin Converting Enzyme (ACE) activity. In this research, we investigate the inhibitory effect of several common edible plants on blocking ACE activity. ACE activity was evaluated by using *N*-hippuryl-L-histidyl-L-leucine (HHL) as substrate and the inhibitory effect of extracts were determined based on the level of hippuric acid by measuring its absorbance using spectrophotometer. Gelatin-salt block test for detection of tannins was carried out prior to the enzymatic assay. Among the extracts tested, *Peperomia pellucida* L. showed strong inhibitory activity with IC_{50} value of 7.17µg/mL, followed by *Nasturtium officinale* and *Sesamum indicum* L. with IC_{50} values of 15.44 and 30.16µg/mL, respectively. Furthermore, *Peperomia pellucida* was the most potent inhibitor of ACE activity *in vitro* with IC_{50} value of 7.17 µg/ml, which was comparable to that of captopril (13.68 µg/ml), a potent ACE inhibitor. This study shows that these plants may develop as antihypertensive agent.

INTRODUCTION: Hypertension is well known as a major risk factor for cardiovascular and renal disease. Asymptomatic pathological condition for years is associated with heart attack or stroke complication in persons with hypertension, so that often called a 'silent killer'. The renin-angiotensin-aldosteron system (RAAS) have an important contribution in maintenance vascular tone and involved in controlling blood pressure. The key enzyme in the RAAS is angiotensin converting enzyme (ACE). ACE is a cell membrane peptidase that plays a central role in the regulation of blood pressure through the production of angiotensin II, the potent vasoconstrictor.

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ACE will catalyse the conversion of angiotensin I into the active vasoconstrictor, angiotensin II [1]. The vasodilating non a peptide bradykinin was also inactivated by ACE, which theoretically contributes to the hypertensive effects of ACE activity [2].

Angiotensin II causes vascular smooth muscle contraction, so the peripheral resistance will increase. Angiotensin II also causes sodium retention (and thereby water retention) in the distal tubules indirectly by stimulates the synthesis and release of aldosterone from the adrenal cortex, which increases blood pressure [2]. Recent research suggests that angiotensin II can inactivates the vasodilatory compounds endothelial derived vascular relaxing factor (nitric oxide – NO) and prostacyclin (PGI2) by stimulates the production of superoxide anion and hydrogen peroxide in the polymorphonuclear leucocytes [3]. From this mechanism can be understood that inhibition of ACE activity will prevent hypertension and its

complication. ACE inhibitors are widely used for the treatment of cardiovascular disease. Since they improve blood pressure, ACE inhibitor can be used to control patients with hypertension and acute myocardial infarction [4], asymptomatic left ventricular dysfunction [5], congestive heart failure [6] and renal dysfunction [7].

Herbal medicine is one of the alternative treatments that are widely used by the people of Indonesia. Indonesia is one of the richest country in the world with natural sources abundance. Many ethno pharmacologic data have been collected based on certain ethnic. Most of them was focus to maintain the vitality and to treat some disease related to age. Based on the daily habit of Indonesia indigenous people, they used to take some wild plants as vegetable and spices, such as *Peperomia pellucida, Eleutherine palmifolia*, *Allium schoenoprassum*, *Sesamum indicum*, *Nasturtium officinale, Limnocharis flava, Luffa cylindrical, Allium cepa, Moringa oleifera, Piper nigrum, Curcuma xanthorrhiza* and *Leucaena leucocephala*. They believed that these plants may give advantage to their health, more specifically on blood flow. Some of plants were also used as traditional medicine since a long time ago to decrease blood pressure. But, the scientific data is limited. We are interested to know the effect of these plants to maintain blood flow associated with blood pressure. As a part of our research for a natural therapeutic approach to the treatment of high blood pressure, *in vitro* screening has been conducted to evaluate the ACE inhibition activity of the methanolic extract of selected medicinal plant in this study.

MATERIALS AND METHODS:

Chemical and Buffer:

The chemicals used in this study were angiotensin converting enzyme(Sigma Aldrich, USA), hippuryl-l-histidyl-l-leucine (Sigma Aldrich, USA), captopril (DexaMedica, Indonesia), hydrochloric acid (Merck, Germany), ethyl acetate (Merck, Germany), natrium hydrochloride (Merck, Germany), potassium dihydrogen phosphate (Merck, Germany).

Plant Materials:

Twelve plants were collected from different parts of Java Island, Indonesia. The voucher specimens were identified and deposited at the Herbarium bogoriense, West Java, Indonesia.

Preparation of Sample:

The plants materials were dried at room temperature and ground to yield powders. One hundred gram of each plant were macerated using methanol (1 x 24 h/each). Each extract was concentrated by using vacuum rotary evaporator to give crude extracts. The yields of extracts were calculated based on dry weight. The solution of plant extracts were prepared by dissolving them in 80% of ethanol. For plant extracts which showed ACE inhibition activity above 80%, serial dilution were made in final concentrations of 100, 50, 25, 12.5 and 6.25 µg/µL.

Enzyme Assay

ACE inhibitor activity was determined by using Cushman and Cheung method with slight modification [8]. Briefly, 20µL of the sample solution was added to 50µL of 8mM HHL as substrate and 10µL of ACE solution (0.25 U/mL). The mixture were mixed well and incubated for 1 hour in 37^{o}C. The reaction was stopped by adding 62.5 µLHCl 1M. The hippuric acid formed was extracted with 375 µL of ethyl acetate. Finally, ethyl acetate layer was dried in vacuum oven and 4mL of water was added. The absorbance of hippuric acid was measured by using UV-Visible spectrophotometer at 228 nm. Blanks were measured by replacing ACE with water while 100% activity value was determined by replacing sample with 20 µL of water.

The percentage inhibition of ACE activity was calculated as follows:

$$\frac{(A-B)-(C-D)}{(A-B)} \times 100 \qquad (1)$$

Where: A = absorbance of control
B = absorbance of control blank
C = absorbance of sample
D = absorbance of control sample

Gelatin-salt Block Test for Detection of Tannins:

Gelatin-salt block test for detection of tannins was carried out according to the method described by Sharifi (2013) with modification. Briefly, 200 mg of extract was dissolved in 4 ml 50°C distilled

water and allowedto cool at room temperature. Four drops of 10%NaCl solution (w/v) was added to the cooledextract to `salt out' any non-tannin compounds, there by eliminating any false-positive tests fortannins [9]. The extract solutions were filtered and 1 ml of the filtrate was placedinto four shallow white porcelain wells. Each well was respectively added with four drops of 1% gelatin solution, four drops of mixture solution containing 1% gelatin and 10% NaCl, and three drops of 10% ferric chloride ($FeCl_3$) solution. No reagent was added to control. The reaction was observed for the formation of precipitate [10].

Phytochemical Screening:

Phytochemical screening was carried out to identify the presence of phytochemical constituent such as alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides and antraquinones. Sample with ACE inhibition activity more than 80% were subjected to phytochemical screening refer to Farnsworth (1996) with slight modification [11].

Statistical Analysis:

All the data are presented as means ± standard deviation (SD) from triplicate experiment and was analysed using Statistically Package for Social Sciences (SPSS) software version 18. A one way analysis of variance (ANOVA) was used for multiple comparison. The concentration of the extracts required to inhibit 50% oxidation (IC_{50}) for active extract was determined using probit programme. $P<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION:

Extraction:

The plants material were successively extracted by maceration using methanol. The crude extracts was obtained from solvent evaporation under vacuum. The yields of crude extracts were calculated based on dry weight. The yields of extract which obtained from maceration technique have a value between 7.38 and 25.32%. Among the extracts, *Allium schoenoprassum* showed the highest yield of extract about 25.32% followed by *Moringa oleifera* L. and *Allium cepa* with the yield values of 21.28 and 15.56%, respectively **(Table 1).**

TABLE 1.THE YIELD OF CRUDE EXTRACTS.

No.	Sample	Part of Plant	The yield of extract (%)
1	*Leucaena leucocephala* (Lam.) de Wit	Seed	7.38
2	*Sesamum indicum* L.	Seed	4.51
3	*Eleutherine palmifolia* L	Rhizome	10.05
4	*Allium schoenoprassum* L.	Leaves	25.32
5	*Moringa oleifera* L..	Leaves	21.28
6	*Peperomia pellucida* (L.) Kunth	Herb	11.15
7	*Nasturtium officinale*	Herb	8.98
8	*Limnocharis flava (L.)*	Leaves	9.50
9	*Luffa cylindrical*	Fruit	10.30
10	*Curcuma xanthorrhiza*Roxb.	Rhizome	7.74
11	*Piper nigrum*	Seed	10.85
12	*Allium cepa* L.	Rhizome	15.56

Activity on ACE Inhibition:

The effect of 12 methanol extracts of selected Indonesia edible plants on ACE activity were evaluated. The inhibitory effect on ACE activity was performed by *in vitro* method using HHL as a substrate. ACE converts HHL into hipuric acid and histidil-leusin. The activity on ACE inhibition was evaluated based on the level of hipuric acid by measuring its absorbance using spectrophotometer. The activity was measured quantitatively in the presence or absence of the extract. Captopril was used as the positive control. Captopril showed high percentage inhibition with value of 86.0% at 100 µg/mL final concentration. The effect of captopril on ACE activity is shown in dose dependent manner. The Inhibition of ACE by captopril at various concentration is shown in **Figure 1**. Captopril is the potent ACE inhibitor for controlling blood pressure. ACE is associated with endothelial dysfunction in hypertension and the development of atherosclerosis. Blockade of ACE activity can prevent the progression of atherosclerosis and reduce the cardiovascular event.

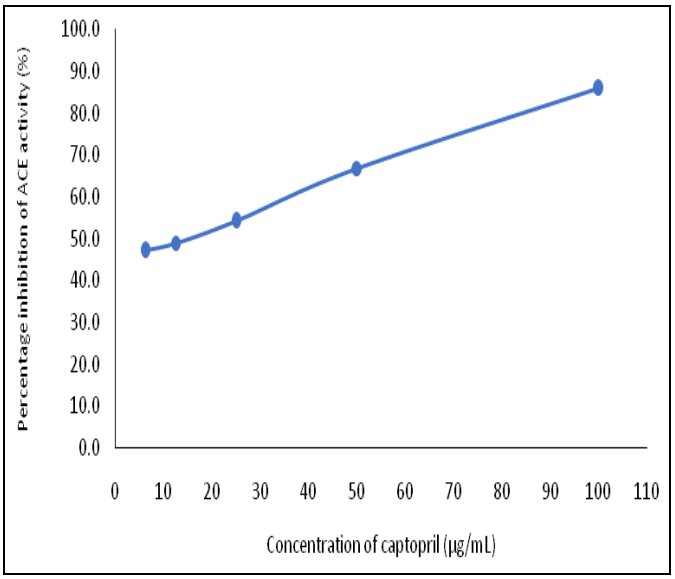

FIGURE 1: INHIBITION OF ACE BY CAPTOPRIL AT VARIOUS CONCENTRATION.

Most of the extracts showed significant inhibition activity at 100 µg/mL final concentration. The results demonstrated that *Sesamum indicum, Peperomia pellucida, Nasturtium officinale, Limnocharis flava, Luffa cylindrica, Curcuma xanthorrhiza* and *Piper nigrum* gave high activity with percentage inhibition of 71.1 to 91.9 at 100µg/mL final concentration **(Fig.2)**. *Nasturtium officinale* showed the most potent activity with percentage inhibition of 91.9. The extracts that showed more than 80% inhibition of ACE activity were further investigated for their effect at various concentrations to obtain the IC_{50} values. The IC_{50} values of the extracts with inhibition of ACE activity are shown in **Table 2**.

The results demonstrated that the extracts showed significant inhibitory activity on ACE with IC_{50} values ranging from 7.17 to 30.16µg/mL. Among the extracts tested, *Peperomia pellucida*, was the strongest inhibitor of ACE activity *in vitro*, followed by *Nasturtium officinale* and *Sesamum indicum*, with IC_{50} values of 7.17, 15.40 and 30.16 µg/mL, respectively. Furthermore, *Peperomia pellucida* was the most potent inhibitor of ACE activity *in vitro* with IC_{50} value of 7.17 µg/mL, which was comparable to that of captopril (13.68 µg/mL), a potent ACE inhibitor.

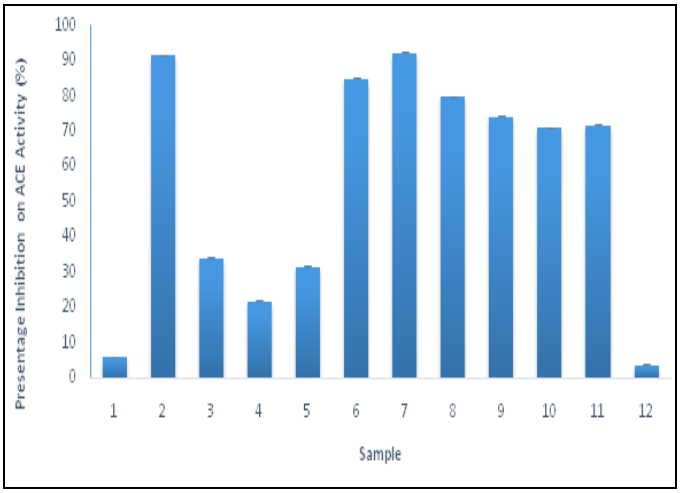

FIGURE 2: PERCENTAGE INHIBITION OF ACE BY SAMPLES AT 100 µg/mL FINAL CONCENTRATION.

Note: 1. *Leucaena leucocephala* (Lam.) de Wit; 2. *Sesamum indicum* L.; 3. *Eleutherine palmifolia* L.; 4. *Allium schoenoprassum* L.; 5. *Moringa oleifera L.*; 6. *Peperomia pellucida (L) Kunth; 7. Nasturtium officinale; 8. Limnocharis flava (L.); 9. Luffa cylindrical; 10. Curcuma xanthorrhiza*Roxb.; 11. *Piper nigrum; 12. Allium cepa* L

TABLE 2.INHIBITION (%) AND IC_{50} VALUES (µg/mL) OF THE METHANOL EXTRACTS OF SOME EDIBLE PLANTS ON ACE INHIBITION.

No.	Sample	Concentration (µg/mL)	Inhibition (%)	IC_{50} (µg/mL)
1	*Leucaena leucocephala (Lam.) de Wit*	100	5.7	
2	*Sesamum indicum* L.	100	91.6*	30.16
		50	66.4	
		25	52.5	
		12.5	37.5	
		6.25	29.4	
3	*Eleutherine palmifolia* L.	100	33.8*	
4	*Allium schoenoprassum* L.	100	21.7*	
5	*Moringa oleifera* L	100	31.5*	
6	*Peperomia pellucida (L.) Kunth*	100	84.9*	7.17
		50	76.1	
		25	63.8	
		12.5	50.1	
		6.25	42.5	
7	*Nasturtium officinale*	100	91.9*	15.40
		50	72.9	

		25	60.2	
		12.5	48.5	
		6.25	38.6	
8	*Limnocharis flava* (L.)	100	79.6*	
9	*Luffa cylindrica*	100	74.1*	
10	*Curcuma xanthorrhiza*Roxb.	100	71.1*	
11	*Piper nigrum*	100	71.3*	
12	*Allium cepa* L.	100	3.5	
13	Captopril	100	86.0*	13.68
		50	66.9	
		25	54.2	
		12.5	48.9	
		6.25	47.0	

*P<0.05,significant difference compared with control

Most of the extracts showed significant inhibition activity which indicate that the extract contained potensial compound as ACE inhibitor. *Peperomia pellucida*, was the strongest inhibitor of ACE activity *in vitro* which was comparable to that of captopril. *Peperomia pellucida* has been used as vegetable in daily life in Indonesia. This plant also was used as traditional medicine since a long time ago to decrease blood pressure. But, the scientific data is limited. From this study, we can conclude that *Peperomia pellucida* showed a mechanism action as ACE inhibitor. Furthermore, it's more potent than captopril as a positive control.

Gelatin-salt Block Test for Detection of Tannins

The gelatin salt test for detection of tannins was done prior to enzyme assay. This test is usefull for determine the presence of tannins which can give false conclusion of activity. Tannin could be found in most of methanolic extracts. Tannins can chelate the zinc ion of ACE, thus inactivating it. In this assay, another chelating agent can interfere decision of conclusion. The result showed tannin contain was limited. There is no significant difference the yield value of extract between before and after tannin elimination. This result showed that the inhibition on ACE activity is not due to tannin contain.

Phytochemical Screening:

Peperomia pellucida, Nasturtium officinale and *Sesamum indicum* were further investigated to determine the source of activity. The common phytochemistry content from plant such as flavonoid, alkaloid, terpenoid and antraquinone have identified **(Table 3)**. Most of recent studies revealed that the inhibitory activity on ACE is due to the flavonoid content in plant. ACE, a crucial enzyme in the regulation of the renin– angiotensin system, is a zinc-containing peptidyl dipeptide hydrolase [12]. The active site of ACE is known to consist of three parts. The first part is a carboxylate binding functionality such as the guanidinium group of arginine.

The second is a pocket that accommodates a hydrophobic side chain of C-terminal amino acid residues and the third is a zinc ion. The zinc ion coordinates to the carbonyl of the penultimate peptide bond of the substrate, whereby the carbonyl group becomes polarized and is subjected to a nucleophilic attack. Flavonoids were suggested to show *in vitro* activity via the generation of chelate complexes within the active centre of ACE [13]. Free hydroxyl groups of phenolic compounds are also suggested to be important structural moieties to chelate the zinc ions. This chelating process will inactivate the ACE [14].

TABLE 3: PHYTOCHEMICAL SCREENING OF THE ACTIVE EXTRACTS.

Phytochemical Constituents	Herb of *Peperomia pellucida*	Herb of *Nasturtium officinale*	Seed of *Sesamum indicum*
Alkaloids	-	+	+
Flavonoids	-	-	-
Saponins	-	+	-
Glycosides	+	-	+
Antraquinones	+	+	-
Terpenoids	+	+	+
Tannins	+	+	-

The interesting result of phytochemical screening of active extracts was founded in this research. There was no flavonoid have been detected.

This results indicate that the active compound for ACE inhibitory activity was not due to flavonoid. Regarding to the results, we suspected terpenoids compound might be the ACE inhibitory compound, since all extracts tested contained terpenoids compound. Recent studies showed that terpenoids also had ACE inhibition activity[15]. Farrugiaet. al. (2013) reported that based on in sillico model, triterpenic molecules (β-amyrin, oleanolic acid and ursolic acid) exhibited binding affinities which are superior to those of captopril, enalaprilat and lisinopril, implying a superior inhibitory activity when compared to the ACE inhibitors that are currently in widespread clinical use. However, alkaloids and glycosides may have responsible for ACE inhibitory activity of these plants since they ever reported possess ACE inhibitory activity[15, 16].

CONCLUSIONS: This study indicates that most of the selected Indonesia edible plants have potential as sources of ACE inhibitor. Further studies will be carried out to identify the active compounds in the plant extracts for development into lead structures with maximum inhibitory activities on ACE activity.

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