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Screening Angiotensin Converting Enzyme (ACE) Inhibitor Activity of Antihypertensive Medicinal Plants from Indonesia

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Research Article

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Abstract

In recent years the use of medicinal plants has been growing worldwide and this is particularly true in Indonesia. Ten parts from nine plants used by traditional healers in Indonesia for the treatment of high blood pressure were investigated for their antihypertensive properties, utilizing the angiotensin converting enzyme assay. Plant materials were macerated successively using petroleum ether, ethyl acetate and methanol. Organic solvent concentrated using rotary vacuum evaporator yield petroleum ether extract (PEE), ethyl acetate extract (EAE) and methanolic extract (ME). ACE inhibitory activity was determined by spectrophotometer and absorbance was read at 228 nm. The results show that leaves and fruits *Phaleria macrocarpa* (Scheff.) Boerl exhibited a strong inhibitory activity against ACE with IC₅₀ in the leaves was 189.13 µg/ml in PEE, 157.74 µg/ml in EEA and 101.52 µg/ml ME, while the IC₅₀ in the fruits was 161.7 µg/ml in PEE, 139.11 µg/ml in the EEA and 122.38 µg/ml in ME.

Keywords: antihypertensive, Indonesian medicinal plants, Angiotensin Converting Enzyme Inhibitors

Introduction

The renin-angiotensin-aldosterone system (RAAS) is a series of coordinated hormonal work. This system controls the cardiovascular system, kidneys and lymph suprarenalis through regulation of fluid and electrolyte balance, and subsequent to the arterial pressure. Setting RAAS on blood pressure via the angiotensin and body fluid balance and electrolytes through aldosterone. Angiotensin II is an

important effectors RAAS hormone and is produced by working on the renin angiotensin I, is then converted to angiotensin II and aldosterone as effectors in SRAA (Karundeng, 2008). One of the drugs used to reduce blood pressure in people with hypertension is ACE inhibitors. ACE inhibitor is the drug of choice for treatment of hypertension and also useful for cardiovascular disease, congestive heart failure disease and left ventricular dysfunction, improving of the arteries, improves endothelial function, remove and stabilize atherosclerotic plaques and in diabetic nephropathy, formation, antioxidant effect and modulate the production of nitric oxide (Engler & Engler, 2004).

Some medicinal plants in Indonesia were used empirically and scientifically proven antihypertensive effect, that are seeds of *Persea americana* Mill (Lauraceae), fruits and leaves of *Phalleria macrocarpa* (Thymeleaceae), leaves of *Oxalis corniculata* (Oxalidaceae), leaves of *Catharanthus roseus* L G Donn (Apocynaceae), *Herbs of Scurulla artopurpurea* (Loranthaceae), seeds of *Swietenia mahogany* (Meliaceae), leaves of *Gynura procumbens* Merr (Compositae), leaves of *Melia azedarach* L (Meliaceae) and leaves of *Hibiscus rosasinensis* L (Malvaceae). However their antihypertensive mechanism have not been elucidated (Imafidon & Amaechina, 2010; Ojewole, *et.al.*, 2007; Anaka, Ozolua, & Okpo, 2009; Yasir Mohammad, Das Sattwik, 2010; Oshimi, *et.al.*, 2008; Ara, Rashid, & Amran, 2009; Perry, 1980; Kim, *et.al.*, 2006; Kate & Lucky, 2010). Hence we interested in doing research on the activity of ACE inhibitors on the plants in search of ACE inhibitors derived from natural materials.

Material and Method

Plants Collection

Plants materials were collected from Bogor, West Java and have been identified in Indonesian Institute of Sciences, Biology Research Centre, Cibinong, Indonesia

Materials

Materials for extraction and fractionation including petroleum ether, ethyl acetate and methanol,



demineralized distilled water (Brataco Chemika, Indonesia), ethyl acetate (Merck, Germany), NaOH, hydrochloric acid (Univar, USA), thin layer chromatography plate of silica gel 60 F 254 (Merck, Germany). Materials for inhibition ACE activity: ACE from rabbit lung (purified ACE), hippuric acid (HA), hippuryl-L-histidyl-L-leucine (HHL), boric acid, NaCl were from Sigma (St. Louis, MO, USA).

Extract Preparation

Plants material were washed in water, dried at 40 °C and powdered. Each material (300 g) was macerated with petroleum ether. Maceration repeated again with the same solvent until the filtrate gives clear maceration results. Maceration results obtained filtered, and the filtrate was concentrated using a rotary vacuum evaporator at a temperature of approximately 50°C to obtain petroleum ether extract (PEE). Residue was macerated by ethyl acetate then filtered and the filtrate was concentrated by vacuum rotary evaporator at a temperature of approximately 50°C to obtain a ethyl acetate extract (EAE), residue from this maceration then macerated with methanol then filtered and the filtrate maceration was concentrated by vacuum rotary evaporator at a temperature of approximately 50°C to obtain extracts (ME). Then each extract were weighed and calculated for yield of extracts.

Phytochemical screening of extracts

Chemical tests were carried out for the PEE, EAE and ME from all materials for the presence of phytochemical constituents like flavonoids, tannins, saponins, terpenoids, alkaloids, glycosides and steroids using thin layer chromatography (TLC) and test tube procedures.

Angiotensin converting enzyme inhibition assay

ACE inhibitory activity was determined by a modification of the method of Cushman and Cheung, 1971 (Cushman & Cheung, 1971; Gao, *et.al*, 2010). The reaction mixture contained 50 µl of 8 mM HHL as a substrate, 100 µl of ACE solution (2,5 mU/ml) and 50 µl of the sample solution. The reaction was carried at 37°C for 90 min, and terminated by addition of 250 µl of 1 N HCl. The hippuric acid formed was extracted with ethyl acetate (1.5 ml), and after removal of ethyl acetate by heat evaporation, hippuric acid was redissolved in aqueduct (3 ml) and the absorbance was measured at 228 nm by Ultrospec 4300 pro UV/visible spectrophotometer. The inhibition activity was calculated using the following equation: Inhibition activity (%) = $[(Ac - As) / (Ac - Ab)] \times 100$

Where, *Ac* is the absorbance of the buffer (control), *As* is the absorbance of the reaction mixture (sample), *Ab* is the absorbance when the stop solution was added before the reaction occurred (blank). The IC₅₀ value was defined as the concentration of extract in mg/ml required to reduce 50% of ACE activity, which was determined by regression analysis of ACE inhibition (%) versus extract concentration.

Results and Discussion

Phytochemical screening

The results of the phytochemical screening of the PEE, EAE and ME using thin layer chromatography and test tube procedures show that its contain flavonoids, tannins, saponins, terpenoids, alkaloids, glycosides and steroids (Table 1).

Table 1: Results of phytochemical screening

Sampel	PEE	EAE	ME
Seeds of <i>Persea americana</i>	Terpenoid	Saponin and tannin	Flavonoid, glikosida, saponin and tannin
Leaves of <i>Phalleria marcocarpa</i>	Glikosida and terpenoid	Flavonoid, glicoside, saponin, tannin and terpenoid	Flavonoid, glikosida, saponin, tannin and terpenoid
Fruit of <i>Phalleria marcocarpa</i>	Terpenoid	Flavonoid, saponin, tannin and terpenoid	Flavonoid, saponin, tannin and triterpenoid
Leaves of <i>Oxalis corniculata</i>	-	Flavonoid, saponin and tannin	Flavonoid, glikosida, saponin, tannin and terpenoid
Leaves of <i>Catharanthus roseus</i>	-	Alkaloid, glikosida and tannin	Flavonoid, alkaloid, glikosida, tannin and steroid
Herbs <i>Scurulla artopurpurea</i>	-	Alkaloid, glikosida and tannin	Flavonoid, alkaloid and tannin
Seeds of <i>Swietenia mahogany</i>	Alkaloid and terpenoid	Alkaloid, saponin and terpenoid	Alkaloid, Flavonoid, saponin, tannin and terpenoid
Leaves of <i>Gynura procumbens</i>	Glikosida	Flavonoid, glikosida and saponin	Flavonoid, glikosida and saponin
Leaves of <i>Melia azedarach</i>	-	Saponin	Flavonoid, glikosida, saponin and terpenoid
Leaves of <i>Hibiscus rosasinensis</i>	Saponin	Flavonoid, saponin and tannin	Flavonoid, alkaloid, glikosida, saponin and tannin

PEE= Petroleum Ether Extracts, EAE = Ethyl Acetate Extracts, ME = Methanol Extracts

Phytochemical constituent from plants has been identified to possess *in vitro* ACE inhibitory activity, including hydrolysable tannins, phenylpropanes, proanthocyanidins, flavonoids, xanthenes, fatty acids, terpenoids, alkaloids, oligosaccharides and peptide amino acids (PJ Park, Je JY, & SK, 2003). However, only a few chemical constituents of plants



that have been studied as the ACE inhibitor mechanism.

Angiotensin converting enzyme inhibition assay

The ACE inhibitory activity of seeds of *Persea americana*, fruits and leaves of *Phalleria marcocarpa*, leaves of *Oxalis corniculata*, leaves of *Catharanthus roseus*, herbs of *Scurulla artopurpurea*, seeds of *Swietenia mahogany*, leaves of *Gynura procumbens*, leaves of *Melia azedarach* and leaves of *Hibiscus rosasinensis* were presented in Table 2 and Figure 1.

Table 2 : ACE inhibitory activity of extracts (IC₅₀)

No	Plants	IC ₅₀ (µg/ml)		
		PEE	EEA	ME
1	Herbs of <i>Scurulla artopurpurea</i>	727	322	380
2	Leaves of <i>Catharanthus roseus</i>	437	1367	402
3	Seeds of <i>Swietenia mahogany</i>	774	1240	1132
4	Seeds of <i>Persea americana</i>	1043	476	500
5	Leaves of <i>Oxalis corniculata</i>	439	325	336
6	Leaves of <i>Phalleria marcocarpa</i>	189	158	102
7	Fruits of <i>Phalleria marcocarpa</i>	161,7	139,11	122,38
8	Leaves of <i>Gynura procumbens</i>	432	227	453
9	Leaves of <i>Melia azedarach</i>	536	588	483
10	Leaves of <i>Hibiscus rosasinensis</i>	431	384	271

Secondary metabolites from plants a natural compound identified as ACE inhibitors are flavonoids, tannins hydrolyzed, xanthons, procyanidins, caffeolyquinic acid. Several studies have shown that many ACE inhibitors are derived from plants, but the identification of the active compounds is still very limited (Balasuriya & Rupasinghe, 2011; Lettre, et al., 2010).

In Indonesia, many herbs have been used to treat hypertension. Some medicinal plants that has antihypertensive activity, that are seeds of *Persea americana*, fruits and leaves of *Phalleria marcocarpa*, leaves of *Oxalis corniculata*, leaves of *Catharanthus roseus*, herbs of *Scurulla artopurpurea*, seeds

of *Swietenia mahogany*, leaves of *Gynura procumbens* Merr, leaves of *Melia azedarach* L and leaves of *Hibiscus rosasinensis* L (Iskandar, 2007; Indonesia Pharmacy Association, 2008).

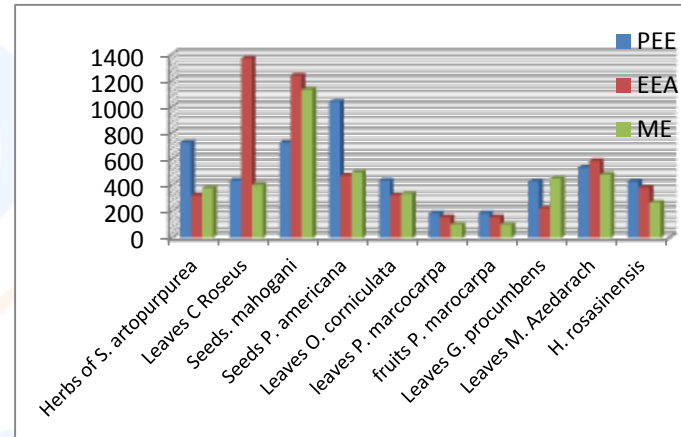


Figure 1: ACE inhibitory activity of extracts (IC₅₀)

Scurulla artopurpure is parasitic on tea plants, but this plant has been used for health. As for the benefits of these plants as medicine include inflammation, hypertension, jaundice, and cancer. *Scurulla Artopurpure* containing variety of chemical compounds such as saponins, tannins, alkaloids and flavonoids (Perry, 1980; Ohashi et al., 2003). The test results showed that herbs of *Scurulla artopurpurea* have ACE inhibitory activity with IC₅₀ value of 727 µg/ml in PEE, 322 µg/ml in the EEA and 380 in the ME.

Catharanthus roseus is widely grown as an ornamental plant. Vinca leaves have antihypertensive effects and efficacious as a cerebral vasodilator (Ara, et al., 2009). Ethanol extract of leaves of vinca has antihypertensive effect and antihyperglycemia. The juice of fresh leaves of vinca has been reported to reduce blood glucose levels in rabbit alloxan-induced diabetes (Nammi, Boini, Lodagala, & Behara, 2003), reduce insulin resistance and antioxidant effect (Rasineni & Desireddy, 2011). Vinca leaf juice can reduce total cholesterol and triglyceride levels in rats (Okokon, 2005). The test results showed that leaves of vinca have ACE inhibitory activity with IC₅₀ value of 437µg/ml in PEE, 1367 µg/ml in EEA, and 402 µg/ml in ME.

Swietenia mahogany has many pharmacological effect as an antidiabetic, antihypertensive, antipyretic, anti-fungal, amobiasis, fever, colds, peptic ulcers and eczema. Mahogany seeds contain flavonoids, tetraterpenoid and fatty acids (Bacsal et al., 1997). Ethanol extract of mahogany seeds reported have antioxidant and xanthin oxidase inhibitor activity (Sahgal et al., 2009). The test results showed that seeds of *Swietenia mahogany* have activity as a weak ACE inhibitory with IC₅₀



value of 774 $\mu\text{g/ml}$ in PEE, 1240 $\mu\text{g/ml}$ EEA and 1132 $\mu\text{g/ml}$ in ME.

Avocado seed (*Persea americana*) empirically used as an antihypertensive. Study of the avocado seeds showed hypotensive effect (Imafidon & Amaechina, 2010; Ojewole *et al.*, 2007). However, the mechanism of the antihypertensive effect of avocado seed is unknown. Besides being used as antihypertensives, several studies have also shown that avocado seed have hypoglycemic and hypolipidemic effect (Koffi, 2009 ; Nwaoguikpe, 2011; Edem, Ekanem, & Ebong, 2009). Chronic hyperglycemia plays a role in the onset of microangiopathy and macroangiopathy that can cause hypertension and renal artery stenosis. At the onset of renal artery stenosis, the juxtaglomerular apparatus release renin which causes the formation of angiotensin I, which is then converted to angiotensin II in the kidney leading to renal vasoconstriction in efferent arterioles. This causes increased pressure on the proximal renal arteries (Yusuf, 2008). The test results showed that seeds of avocado have ACE inhibitory activity with IC_{50} values 1043 $\mu\text{g/ml}$ in EE, 476 $\mu\text{g/ml}$ in EEA and 500 $\mu\text{g/ml}$ in ME.

Oxalis corniculata reported has antihypertensive effects, hypoglycemic effect, uterine relaxants, antipsychotics, CNS stimulants, muscle relaxants plain and antibacterial activity (Sakat, Juvekar, & Gambhire, 2010). Methanolic extract of *Oxalis corniculata* has antioxidant activity and contains flavonoids, alkaloids, terpenoids, saponins, glycosides, and steroids phlobatanin. Total phenol content was estimated as 25.62 mg of gallic acid equivalents of dry extract. Total flavonoids and flavonols were found to be 150.88 and 150.16 mg of rutin equivalents per gram of dry extract respectively (Sakat *et al.*, 2010) (Khan & Zehra, 2011). The test results showed that leaves of *Oxalis corniculata* have ACE inhibitory activity with IC_{50} value of 439 $\mu\text{g/ml}$ in PEE, 324 $\mu\text{g/ml}$ in EEA and 336 $\mu\text{g/ml}$ in ME.

Phaleria macrocarpa is native to Indonesia, efficacious as a medicine. Leaves and fruits of *Phaleria macrocarpa* empirically been used to treat various types of diseases such as cancer, liver disorders, heart disease, diabetes, arthritis, kidney disorders, stroke, and high blood pressure (Harmanto, 2003). The use of *Phaleria macrocarpa* traditional medicine is very likely due to leaf and fruit of *Phaleria macrocarpa*. contains chemical compounds such as alkaloids, saponins, polyphenols, and fruit contained alkaloids and saponins also contain flavonoids (Sumastuti.R, 2003). *Phaleria macrocarpa* contains mahkotaside, mangiferin, kaempferol-3-od glucoside, dodecanoat acid, palmitic acid, ethyl stearate and sucrose (Oshimi *et al.*, 2008). The content of lignans in *Phaleria macrocarpa* (Scheff.) Boerl are pinoresinol, lariciresinol and matairesinol (Saufi. A., *et.al*, 2008). The results showed that *Phaleria macrocarpa* (Scheff.) Boerl has antidiabetic effects that inhibit the enzyme alpha-glukosidase and have anti-diabetic effect in mice induced sterptozotosin. The test results showed that the leaves and fruits of *Phaleria macrocarpa* have ACE inhibitory activity with IC_{50} values in the leaves was 189 $\mu\text{g/ml}$ in PEE 158 $\mu\text{g/ml}$ in EEA and 102 $\mu\text{g/ml}$ ME. While the IC_{50} values in the fruit was 162 $\mu\text{g/ml}$ in PEE, 139 $\mu\text{g/ml}$ in the EEA and 122 $\mu\text{g/ml}$ in ME.

In Indonesia *Gynura procumbens* is used for the treatment of various diseases such as hypertension, vasodilators, fever, diabetes hyperlipidemia and diabetes mellitus mellitus (Perry, 1980). Water extract of *Gynura procumbens* is effective in reducing blood pressure, lactate dehydrogenase, creatinine phosphate kinase and increases nitric oxide in mice (Kim *et al.*, 2006). Ethanollic extract of leaf *Gynura procumbens* can reduce serum triglyceride and cholesterol in diabetes induced sterptozotosin, antidiabetic effects comparable to biguanid (June *et al.*, 2012). Diabetic effect of *Gynura procumbens* mediated through increased metabolism of glucose via the glycolytic pathway and inhibits production glucose endogenous in liver via gluconeogenesis pathway (Lee, Hakim, Rabu, & Sani, 2012). The test results showed that leaves of *Gynura procumbens* have ACE inhibitory activity with IC_{50} values 432 $\mu\text{g/ml}$ in PEE 227 $\mu\text{g/ml}$ in EEA and 453 $\mu\text{g/ml}$ in ME.

Melia azedarach has been used empirically for the treatment of hypertension, ascariasis, oxyuriasis, taeniasis, trichuriasis, scabies and fungus on the scalp (*Tinea capitis*), stomach and abdominal pain (Anonim, 2011). *Melia azedarach* contain active compoud such as flavonoid, tannins, saponins, steroids/ triterpenoids, sodium, calcium, potassium, magnesium and the compound of phenolic acids and ferulic acid (Hariana, 2006). The test results showed that the leaves of *Melia azedarach* have ACE inhibitory activity with IC_{50} value 536 $\mu\text{g/ml}$ in PEE, 588 $\mu\text{g/ml}$ in EEA and 483 $\mu\text{g/ml}$ in ME.

Hibiscus rosasinensis has efficacy as antihypertensive, fever in children, cough medicine and thrush. Leaves of *H. rosasinensis* in Nigeria has been used by people as an addition to the vitality of a man (aprodisiaca) (Sreenivas, 2011). Kate *et.al*, 2010, have proven that the water extract of *Hibiscus rosasinensis* leaves have hypertensive effect in rats (Kate & Lucky, 2010). The test results showed that the leaves of *Hibiscus rosasinensis* have ACE inhibitory activity with IC_{50} value 430.52 $\mu\text{g/ml}$ in PEE, 383.51 $\mu\text{g/ml}$ in EEA and 270.8 ppm in ME.

Conclusion

The present study suggests that seeds of *Persea americana*, fruits and leaves of *Phaleria macrocarpa*, leaves of *Oxalis corniculata*, leaves of *Catharanthus roseus*, Herbs of *Scurulla artopurpurea*, seeds of *Swietenia mahogany*, leaves of *Gynura procumbens*, leaves of *Melia azedarach* L and leaves of *Hibiscus rosasinensis* possess angiotensin converting enzyme inhibitory that might be helpful in treating hypertension. Further investigations on the isolation of active compounds present in the extract and *in vivo* studies are necessary to identify a potential chemical entity for



clinical use in the treatment of hypertension and other related cardiovascular disorders.

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.