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

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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 methanol 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 EAE 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 the 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 antyhypertensive 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 using a rotary vacuum evaporator at a temperature of approximately 50°C to obtain an 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 (%) = \([Ac\text{–}As / (Ac\text{–}Ab)] \times 100\)

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 IC_{50} 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**

<table>
<thead>
<tr>
<th>Sample</th>
<th>PEE</th>
<th>EAE</th>
<th>ME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seeds of *Persea americana*</td>
<td>Terpenoid</td>
<td>Saponin and tannin</td>
<td>Flavonoid, glikosida, saponin and tannin</td>
</tr>
<tr>
<td>Leaves of <em>Phaleria marocarpa</em></td>
<td>Glikosida and terpenoid</td>
<td>Flavonoid, glikosida, saponin, and terpenoid</td>
<td>Flavonoid, glikosida, saponin, and tannin</td>
</tr>
<tr>
<td>Fruit of <em>Phaleria marocarpa</em></td>
<td>Terpenoid</td>
<td>Flavonoid, saponin, and terpenoid</td>
<td>Flavonoid, glikosida, saponin, and tannin</td>
</tr>
<tr>
<td>Leaves of <em>Oxalis corniculata</em></td>
<td>-</td>
<td>Flavonoid, saponin and tannin</td>
<td>Flavonoid, glikosida, saponin, and tannin</td>
</tr>
<tr>
<td>Leaves of <em>Catharanthus roseus</em></td>
<td>-</td>
<td>Alkaloid, glikosida and tannin</td>
<td>Flavonoid, alkaloid, glikosida, and tannin</td>
</tr>
<tr>
<td>Herbs <em>Scutella artopurpurea</em></td>
<td>-</td>
<td>Alkaloid, glikosida and tannin</td>
<td>Flavonoid, alkaloid, glikosida, and tannin</td>
</tr>
<tr>
<td>Seeds of <em>Swietenia mahogany</em></td>
<td>Alkaloid and terpenoid</td>
<td>Alkaloid, saponin and terpenoid</td>
<td>Alkaloid, Flavonoid, saponin, and tannin</td>
</tr>
<tr>
<td>Leaves of <em>Gynura procumbens</em></td>
<td>Glikosida</td>
<td>Flavonoid, glikosida and saponin</td>
<td>Flavonoid, glikosida and saponin</td>
</tr>
<tr>
<td>Leaves of <em>Melia azedarach</em></td>
<td>-</td>
<td>Saponin</td>
<td>Flavonoid, glikosida, saponin and terpenoid</td>
</tr>
<tr>
<td>Leaves of <em>Hibiscus rosasinensis</em></td>
<td>Saponin</td>
<td>Flavonoid, saponin and tannin</td>
<td>Flavonoid, alkaloid, glikosida, and saponin</td>
</tr>
</tbody>
</table>

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, xanthones, 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* Merr, leaves of *Melia azedarach* L and leaves of *Hibiscus rosasinensis* were presented in Table 2 and Figure 1.

**Table 2**: ACE inhibitory activity of extracts (IC$_{50}$)

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

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 have 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* have been used for health. As for the benefits of these plants as medicine include inflammation, hypertension, jaundice, and cancer. *Scurulla Artopurpure* containing various 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 IC$_{50}$ 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 antihiperglicemia. 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 IC$_{50}$ 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, tetratriterpenoid 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 IC$_{50}$...
value of 774 µg/ml in PEE, 1240 in µg/ml EEA and 1132 µg/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 hyperlipidemic 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 IC₅₀ values 1043 µg/ml in EE, 476 µg/ml in EEA and 500 µg/ml in ME.

Oxalis corniculata reported has antihypertensive effects, hypoglycemic effect, hypotensive effect, uterine relaxants, antipsychotics, CNS stimulants, muscle relaxants 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 IC₅₀ value of 439 µg/ml in PEE, 324 µg/ml in EEA and 336 µg/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-3-0d glucoside, dodecanoat acid, palmitic acid, ethyl stearate and sucrose (Oshimi et al., 2008). The content of lignans in Phaleria macrocarpa (Scheff.) Boeri are pinoresinol, lariciresinol and matairesinol (Saufi. A., et al., 2008). The results showed that Phaleria macrocarpa (Scheff.) Boeri has anti-diabetic effects that inhibit the enzyme alpha-glukosidase and have anti-diabetic effect in mice induced streptozotocin. The test results showed that the leaves and fruits of Phaleria macrocarpa have ACE inhibitory activity with IC₅₀ values in the leaves was 189 µg/ml in PEE 158 µg/ml in EEA and 102 µg/ml ME. While the IC₅₀ values in the fruit was 162 µg/ml in PEE, 139 µg/ml in the EEA and 122 µg/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). Ethanolic extract of leaf Gynura procumbens can reduce serum triglyceride and cholesterol in diabetes induced streptozotocin, 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 IC₅₀ values 432 µg/ml in PEE 227 µg/ml in EEA and 453 µg/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 compound 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 IC₅₀ value 536 µg/ml in PEE, 588µg/ml in EEA and 483 µg/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 (aprodiasiaca) (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 IC₅₀ value 430.52 µg/ml in PEE, 383.51 µg/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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References


**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.