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

by Aprilita Rina Yanti Eff

Submission date: 22-Oct-2019 06:29PM (UTC+0700)
Submission ID: 1197955939
File name: B20.pdf (331.84K)
Word count: 4024
Character count: 23177

International Journal of Pharmacy Teaching & Practices 2013, Vol.4, Issue 1, 527-532.

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

Aprilita Rinayanti [1*], Maksum Radji[2], Abdul Mun'im [2]and F.D. Suyatna[3]
[1]Faculty of Pharmacy, Tujuh Belas Agustus 1945 University, Jakarta, Indonesa.
[2]Faculty of Pharmacy University of Indonesia, Depok, 16424, Indonesia.
[3]Faculty of Medicine, University of Indonesia, Salemba, Jakarta, Indonesia.

Research Article

Please cite this paper as: Aprilita Rinayanti [1*], Maksum Radji[2], Abdul Mun'im [2]and F.D. Suyatna[3]. Screening Angiotensin Converting Enzyme (ACE) Inhibitor Activity of Antihypertensive Medicinal Plants from Indonesia. IJPTP, 2013, 4(1), 527-532.

Corresponding Author:

Aprilita Rinayanti
Faculty of Pharmacy, Tujuh Belas Agustus 1945 University, Jakarta, Indonesa
Email: *aprilita.rinayanti@gmail.com*
Phone no: 08129939727

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 vacum evaporator yield petroleum ether extract (PEE), ethyl acetate extract (EAE) and methanolic extract (ME). ACE inhibitory activity was determined by spechtrophotometer 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 IC_{50} in the leaves was 189.13 µg/ml in PEE, 157.74 µg/ml in EEA and 101.52 µg/ml ME, while the IC_{50} 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 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 marcocarpa* (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,

deminelarized 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 (purifed 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^{0}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^{0}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 50^0 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 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

Phytochemichal 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).

Tabel 1: Results of phytochemical screening

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

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

Table 2 : ACE inhibitory activity of extracts (IC_{50})

No	Plants	IC_{50} (µg/ml)		
		PEE	EEA	ME
1	Herbs *of Scurulla artopurpurea*	727	322	380
2	Leaves of *Catharanthus roseus*	437	1367	402
3	Seeds of *Swietenia mahogany*	774	1240	1132
4	Seeds of *Persea americana*	1043	476	500
5	Leaves of *Oxalis corniculata*	439	325	336
6	Leaves of *Phalleria marcocarpa*	189	158	102
7	Fruits of *Phalleria marcocarpa*	161,7	139,11	122,38
8	Leaves of *Gynura procumbens*	432	227	453
9	Leaves of *Melia azedarach*	536	588	483
10	Leaves of *Hibiscus rosasinensis*	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 Assocciation, 2008).

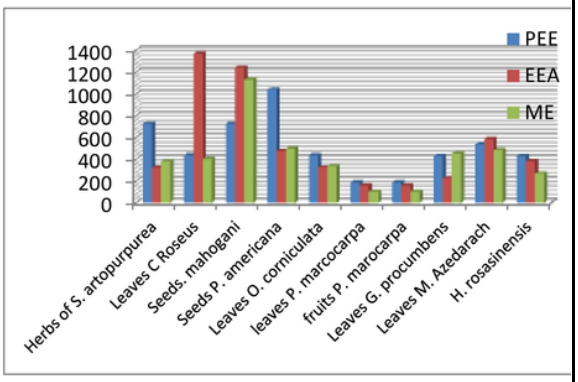

Figure 1: ACE inhibitory activity of extracts (IC_{50})

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 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 hypolipidemic effect (Koffi, 2009 ; Nwaoguikpe, 2011; Edem, Ekanem, & Ebong, 2009). Chronic hyperglycemia plays a role in the onset of microanghiopaty and macroanghiopaty 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 µg/ml in EE, 476 µg/ml in EEA and 500 µg/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 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-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 µg/ml in PEE 158 µg/ml in EEA and 102 µg/ml ME. While the IC_{50} 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 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 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 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 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 (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 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 *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* 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.

Acknowledgement

This research was supported by funds from the Madya Research Grant – University of Indonesia 2013.

References

1. Anaka, O. N., Ozolua, R. I., & Okpo, S. O. (2009). Effect of the aqueous seed extract of *Persea americana* mill (Lauraceae) on the blood pressure of *Sprague dawley* rats. *African Journal of Pharmacy and Pharmacology*, *3*(10), 485–490.

2. Ara, N., Rashid, M., & Amran, S. (2009). Comparison of hypotensive and hypolipidemic effects of *Catharanthus roseus* leaves extract with atenolol on adrenaline induced hypertensive rats. *Pakistan Journal of Pharmaceutical Science*, *22*(3), 267–271.

3. Bacs, Chavez.L, Espina.S, Javillo.J, & Manzanilla.H. (1997). The Effect of *Swietenia Mahogani* (Mahogany) Seed Extracts On Indomethacin-Inducd Gastric Ulcers In Female Sprague-Dawley. *Acta Medica Phillippina*, *3*, 127–139.

4. Balasuriya, B. W. N., & Rupasinghe, H. P. V. (2011). Plant flavonoids as angiotensin converting enzyme inhibitors in regulation of hypertension. *Functional Foods in Health and Disease*, *5*, 172–188.

5. Cushman, D. W., & Cheung, H. S. (1971). Spectrophotometric assay and properties of the Angiotensin Converting Enzyme of rabbit lung. *Biochemical pharmacology*, *20*(7), 1637–48.

6. Edem, D. O., Ekanem, I. S., & Ebong, P. E. (2009). Effect of aqueous extracts of alligator pear seeds (*Persea americana)* Mill on blood glucose and histopathology of pancreas in alloxan induced diabetic rats. *Pakistan Journal of Pharmaceutical Science*, *22*(3), 272–276.

7. Engler, M. B., & Engler, M. M. (2004). The vasculoprotective effects of flavonoid-rich cocoa and chocolate. *Nutrition Research*, *24*(9), 695–706.

8. Gao, D., Chang, T., Li, H., & Cao, Y. (2010). Angiotensin I-converting enzyme inhibitor derived from cottonseed protein hydrolysate. *African Journal of Biotechnology*, *9*(53), 8977–8982.

9. Hariana. (2006). *Medicinal Plants and Usefulness*. Penebar Swadaya Jakarta.

10. Harmantoo. (2003). Research against Leaves and Fruit Crown god. *One Day Seminar Mahkota Dewa*. Center for Research and Development of Pharmaceutical and Traditional Medicine, Ministry of Health of the Republic of Indonesia.

11. Indonesia Pharmaceuticals Association, Board of Central Java. (2008). Indonesia Natural Medicines List (DOA) (III., pp. 55–57).

12. Imafidon, K. E., & Amaechina, F. C. (2010). Effects of Aqueous Seed Extract of *Persea americana* Mill . (Avocado) on Blood Pressure and Lipid Profile in Hypertensive Rats. *Advances in Biological Research*, *4*(2), 116–121.

13. Iskandar, Y. (2007). Medicinal plants as an antihypertensive effect. *Scientific Work*, 1–27.

14. June, C. C., Wen, H., Ani, H. A., Latif, J., & Gansau, J. A. (2012). Hypoglycemic Effects of *Gynura procumbens* Fractions on Streptozotocin-induced Diabetic Rats involved Phosphorylation of GSK3β (Ser-9) in Liver. *Sains Malaysiana*, *41*(8), 969–975.

15. Karundeng, R. (2008). Role of Renin Renin Angiotensin Aldosterone system (biological effects of Renin). *BIK Biomedic*, *4*(3), 106–113.

16. Kate, I. E., & Lucky, O. O. (2010). The effects of aqueous extracts of the leaves of *Hibiscus rosasinensis* Linn . on renal function in hypertensive rats. *African Journal of Biochemistry Research*, *4*(2), 43–46.

17. Khan, R. M., & Zehra, H. (2013). Amelioration of CCl4-induced nephrotoxicity by *Oxalis corniculata* in rat. *Experimental Toxicologic and Pathology*, 66 (3), 327–34..

18. Kim, M., Lee, H. J., Wiryowidagdo, S., & Kim, H. K. (2006). Antihypertensive Effects of *Gynura procumbens* Extracts in Spontaneusly Hypertensive Rats. *Journal of Medical food*, *9*(4), 587–590.

19. Koffi, N. (2009). Effect of Aqueous Extract of *Persea Americana* Seeds on the Glycemia of Diabetic Rabbits, European Journal of Scientific Research *26*(3), 376–.

20. Lee, H., Hakim, P., Rabu, A., & Sani, H. A. (2012). Antidiabetic effect of *Gynura procumbens* leaves extracts involve modulation of hepatic carbohydrate metabolism in streptozotocin-induced diabetic rats. *Journal of Medicinal Plants Research*, *6*(5), 796–812.

21. Lettre, D. P., Kumar, R., Kumar, A., Sharma, R., & Tuwa, A. (2010). Pharmacological review on Natural ACE inhibitors. *Der Pharmacia Lettre*, *2*(2), 273–293.

22. Nammi, S., Boini, M. K., Lodagala, S., & Behara, R. (2003). The Juice of Fresh Leaves of Catharanthus roseus Linn. Reduces Blood Glucose in Normal and Alloxan Diabetic Rabbits. *BMC Complementary and Alternative Medicine*, *2*, 3–4.

23. Nwaoguikpe, R. N. (2011). The effect of aqueous seed extract of *Persea americana* (avocado pear) on serum lipid and cholesterol levels in rabbits. *African Journal of Pharmacy and Pharmacology Research*, *1*(2), 23–29.

24. Ohashi, K., Winarno, H., Mukai, M., Inoue, M., Hana, M. S., Simanjuntak, P., & Shibuya, H. (2003). Indonesian medicinal plants. XXV. Cancer cell invasion inhibitory effects of chemical constituents in the parasitic plant *Scurrula atropurpurea* (Loranthaceae). *Chemical & pharmaceutical bulletin*, *51*(3), 343–5.

25. Ojewole, J. A. O., Gondwe, M. M., Moodley, K., & Musabayane, C. T. (2007). Cardiovascular Topics Cardiovascular effects of *Persea americana* Mill (Lauraceae) (avocado) aqueous leaf extract in

experimental animals. *Cardiovascular Journal of South Africa*, *18*(2), 69–76.

26. Okokon, B. S. A. and J. E. (2005). Effect of leaf juice of *Catharanthus roseus* Linn on cholesterol , cholesterol , triglyceride and lipoproteins levels in normal rats rats. *Indian Journal Pharmacology*, *37*(6), 401–402.

27. Oshimi, S., Zaima, K., Matsuno, Y., Hirasawa, Y., Iizuka, T., Studiawan, H., Indrayanto, G., et al. (2008). Studies on the constituents from the fruits of *Phaleria macrocarpa*. *Journal of natural medicines*, *62*(2), 207–10.

28. Perry, L. (1980). *Medicinal Plants of East and Southeast Asia□: Attributated Properties and Uses* (pp. 94–95). MIT Press, Cambridge. MA.

PJ Park, Je JY, & SK, K. (2003). Angiotensin I converting enzyme (ACE) inhibitory activity of hetero-chitooligosaccharides prepared from partially different deacetylated chitosans. *Journal of Agriculture Food Chemistry*, *51*, 4930–4934.

30. Rasineni, K., & Desireddy, S. (2011). Preventive effect of *Catharanthus roseus* (Linn.) against high fructose diet-induced insulin resistance and oxida- tive stress in male Wistar rats. *Journal of Diabetes Mellitus*, *1*(3), 63–70.

31. Sahgal, G., Ramanathan, S., Sasidharan, S., Mordi, M. N., Ismail, S., & Mansor, S. M. (2009). In-vitro antioxidant and xanthine oxidase inhibitory activities of methanolic *Swietenia mahagoni* seed extracts. *Molecules (Basel, Switzerland)*, *14*(), 4476–85.

32. Sakat, S. S., Juvekar, A. R., & Gambhire, M. N. (2010). In-vitro antioxidant and antiinflammatory activity of methanol extract of *Oxalis corniculata* linn. *International Journal of Pharmacy and Pharmaceutical Sciences*, *2*(1), 146–155.

33. Saufi. A., Heimendahl.C.B, V., Alfermann.A.W., & Fuss. (2008). Stereochemistry of lignans in *Phaleria macrocarpa* (Scheff.) Boerl. *Naturforsch*, *63*(), 13–16.

34. Sreenivas, R. M. and S. A. (2011). Anti-Diabetic Effects of Aqueous Ethanolic Extract of *Hibiscus rosasinensis* L.on Streptozotosin-Induced Diabetic Rats and the Possible Morphological Changes in the Liver and kidney. *International Journal of Pharmacology*, *7*(3), 363–369.

35. Sumastuti.R. (2003). Research against Leaves and Fruit Crown god. One Day Seminar Mahkota Dewa. Center for Research and Development of Pharmaceutical and Traditional Medicine, Ministry of Health of the Republic of Indonesia.

36. Yasir Mohammad, Das Sattwik, K. M. K. (2010). The Phytochemical and Pharmacological Profile of *Persea americana* Mill. *Pharmacog Review*, *4*, 77–84.

37. Yusuf, I. (2008). Secondary hypertension . *Medical Review*, *21*(3).

AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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

ORIGINALITY REPORT

15%	8%	13%	7%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Subish Palaian. "Pharmacovigilance Practices and Activities", Elsevier BV, 2018 Publication	1%
2	Submitted to Unika Soegijapranata Student Paper	1%
3	KAU Samanthi, S Wickramarachchi, EMK Wijeratne, PA Paranagama. "Two new bioactive polyketides from Curvularia trifolii, an endolichenic fungus isolated from Usnea sp., in Sri Lanka", Journal of the National Science Foundation of Sri Lanka, 2015 Publication	1%
4	Djamila Meriane, Grégory Genta-Jouve, Mohamed Kaabeche, Sylvie Michel, Sabrina Boutefnouchet. "Rapid Identification of Antioxidant Compounds of Genista saharae Coss. & Dur. by Combination of DPPH Scavenging Assay and HPTLC-MS", Molecules, 2014 Publication	1%

5	HAIKUAN WANG. "An application in Gouda cheese manufacture for a strain of Lactobacillus helveticus ND01", International Journal of Dairy Technology, 08/2011 Publication	1%
6	Mallaiah Putakala, Sudhakara Gujjala, Srinivasulu Nukala, Saralakumari Desireddy. "Beneficial Effects of Phyllanthus amarus Against High Fructose Diet Induced Insulin Resistance and Hepatic Oxidative Stress in Male Wistar Rats", Applied Biochemistry and Biotechnology, 2017 Publication	1%
7	www.iconceptpress.com Internet Source	1%
8	riubu.ubu.es Internet Source	1%
9	pankajinprintmedia.blogspot.com Internet Source	1%
10	fr.scribd.com Internet Source	1%
11	Darussalam, Handry, Titik Nuryastuti, . Mursiti, and . Mustofa. "Antimicrobial activity of bioactive compounds isolated from Swietenia mahagoni (L) Jacq. against Staphylococcus aureus and Pseudomonas aeruginosa", Journal of thee	1%

Medical Sciences (Berkala Ilmu Kedokteran), 2014.
Publication

---

12 www.biotek.gov.my 1%
Internet Source

---

13 Andrew C Duncan, Anna K Jäger, Johannes van Staden. "Screening of Zulu medicinal plants for angiotensin converting enzyme (ACE) inhibitors", Journal of Ethnopharmacology, 1999 1%
Publication

---

14 Arunsi UO. "Sub-Lethal Assessment of Aqueous Dried Leaf Extract of Catharanthus roseus (Linn.) G. Don in Male Albino Rats", MOJ Toxicology, 2017 1%
Publication

---

15 Zouari, N.. "Chemical composition, angiotensin I-converting enzyme inhibitory, antioxidant and antimicrobial activities of essential oil of Tunisian Thymus algeriensis Boiss. et Reut. (Lamiaceae)", Food and Bioproducts Processing, 201110 1%
Publication

---

16 Jung, W.K.. "Effect of chitooligosaccharides on calcium bioavailability and bone strength in ovariectomized rats", Life Sciences, 20060125 <1%
Publication

Maria Inés Torino, Rocío I. Limón, Cristina

17	Martínez-Villaluenga, Sari Mäkinen et al. "Antioxidant and antihypertensive properties of liquid and solid state fermented lentils", Food Chemistry, 2013 Publication	<1%
18	eprints.ums.edu.my Internet Source	<1%
19	Submitted to Acadia University Student Paper	<1%
20	Submitted to University of Hong Kong Student Paper	<1%
21	www.j3.jstage.jst.go.jp Internet Source	<1%
22	nurseweb.ucsf.edu Internet Source	<1%
23	Submitted to Florida State University Student Paper	<1%
24	Jae-Young Oh, Eun-A Kim, Hyogeun Lee, Hyun-Soo Kim, Jung-Suck Lee, You-Jin Jeon. "Antihypertensive effect of surimi prepared from olive flounder (Paralichthys olivaceus) by angiotensin-I converting enzyme (ACE) inhibitory activity and characterization of ACE inhibitory peptides", Process Biochemistry, 2019 Publication	<1%

25	Iwaniak, Anna, Piotr Minkiewicz, and Małgorzata Darewicz. "Food-Originating ACE Inhibitors, Including Antihypertensive Peptides, as Preventive Food Components in Blood Pressure Reduction : Food peptides reducing blood pressure…", Comprehensive Reviews in Food Science and Food Safety, 2014. Publication	<1%
26	Submitted to Institute of Research & Postgraduate Studies, Universiti Kuala Lumpur Student Paper	<1%
27	krishikosh.egranth.ac.in Internet Source	<1%
28	Muhammad Kamal Ja'afar, Shajarahtunnur Jamil, Norazah Basar, Mohd Bakri Bakar et al. " Lactones and Flavonoids isolated from the Leaves of ", Natural Product Communications, 2017 Publication	<1%
29	central.oak.go.kr Internet Source	<1%
30	Anna Jakubczyk, Michał Świeca, Urszula Gawlik-Dziki, Dariusz Dziki. "Nutritional potential and inhibitory activity of bread fortified with green coffee beans against enzymes involved in metabolic syndrome pathogenesis", LWT, 2018 Publication	<1%

31	www.arjournals.org Internet Source	<1%

Exclude quotes	On	Exclude matches	< 10 words
Exclude bibliography	Off		