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Original Article

ACE Inhibitory Activity, Total Phenolic and Flavonoid Content of *Pereskia saccharose* Griseb. Leaves Extract

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ABSTRACT

Introduction: Angiotensin-converting enzyme inhibitors (ACEi) are drugs that can control hypertension. *Pereskia saccharose* Griseb. leaves have been used traditionally as antihypertensive. **Objective:** The objective of this study was to determine the antihypertensive activity through inhibition of ACE activity, the total phenolic content and total flavonoid content of the ethanolic extract of *Pereskia saccharose* Griseb. leaves and its fractions. **Methods:** Extraction was done by maceration with 80% ethanol and fractionation performed by liquid-liquid partition. **Results:** *In vitro* ACE inhibitory activity assay of the ethanolic extract using ACE Kit-WST Dojindo had IC_{50} value of 3.448 µg/mL and ethyl acetate fraction had IC_{50} value of 1.714 x 10-3 µg/mL. Ethyl acetate contained the highest amounts of both TPC (72.991 ± 0.932 mg GAE/g sample) and TFC (61.337 ± 1.612 mg QE/g sample). **Conclusion:** The results suggest that Pereskia saccharose Griseb. possess ACE inhibitory activity.

Key words: ACE inhibitor, Flavonoid, *Pereskia saccharose* Griseb, Phenolic.

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INTRODUCTION

Hypertension has become one of the biggest health problems in the world because it has a high prevalence and has been associated with the increased risk of cardiovascular and kidney disease.[1] Based on the WHO (2013), the global prevalence of hypertension was 40% and in 2025 is predicted as much as 60% of the total world population will suffer from hypertension.[1,2]

Antihypertensive drugs are needed to control blood pressure of hypertensive patients. Angiotensin-converting enzyme inhibitors (ACEi) are drugs to control hypertension. Angiotensin-converting enzyme (ACE) plays a role in converting angiotensin I to angiotensin II, which is responsible for triggering the mechanism of increased blood pressure.[3]

Many explorations on medicinal plants that can be used as an alternative treatment of hypertension have been done. Secondary metabolites in medicinal plants have been reported to have an inhibitory effect on ACE activity, including flavonoids, terpenoids, alkaloid, proanthocyanidin, hydrolyzed tannins, fatty acids, peptides, and xanthones.[4]

Pereskia saccharose Griseb. is one of the plants that has potential as a medicinal plant. Its leaves can be eaten raw or taken as a concoction brewed from fresh plant. Recent studies showed that *Pereskia saccharose* Griseb. leaves have been used empirically by the public as an antihypertensive.[5] However, so far no studies have proven the efficacy and the antihypertensive mechanism of these plants scientifically. The objective of this study was to determine the antihypertensive activity through inhibition of ACE activity, the total phenolic content and total flavonoid content of the ethanolic extract of *Pereskia saccharose* Griseb. leaves and its fractions in solvents of different polarities.

MATERIALS AND METHODS

Materials

ACE Kit-WST A502 (Dojindo, Japan), *Pereskia saccharose* Griseb. leaves, captopril (Kimia Farma, Indonesia), gallic acid (Sigma-Aldrich, USA), quercetin (Sigma-Aldrich, USA), Folin-Ciocalteu (Merk, Germany), ethanol, n-hexane, ethyl acetate, and n-butanol (BRATACO, Indonesia). All other reagents were analytical grades.

Preparation of Sample

Plants materials were collected from Bogor, Indonesia and have been identified at Indonesian Institute of Sciences, Bogor, Indonesia. The leaves were thoroughly washed with tap water, sorted, cut, dried, and grinded into powder. Five hundred grams of powdered samples were extracted by maceration using 80% of ethanol (7 x 2.5 L). The extracts were combined and concentrated using vacuum rotary evaporator to give crude extracts and calculated for a yield of extracts. Fifty grams of the crude extract were suspended in distilled water (500 mL) and partitioned successively with n-hexane, ethyl acetate, and n-butanol. The solvents were removed with vacuum rotary evaporator to produce dried fractions.

Determination of ACE Inhibitory Activity

The determination of ACE inhibitory activity was measured by a colorimetric method using ACE kit-WST (Dojindo, Japan) according to the manufactur-

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er's protocol. The absorbance was read at 450 nm using PG Instruments Ltd. T80 microplate reader. Inhibition activity of ACE by the sample calculated according to the formula written on the manual procedure. The IC_{50} value was defined as the concentration of sample in µg/mL required to reduce 50% of ACE activity, which was determined by regression analysis of ACE inhibition (%) versus extract concentration.[6]

Determination of Total Phenolic Content (TPC)

Total phenolic content was measured using the modified Folin-Ciocalteu method adapted from Al-Saeedi and Hossain.[7] The absorbance was read at wavelength 740 nm. The analysis was done in triplicate. The total phenolic content was reported as total gallic acid equivalent per g sample (mg GAE/g).

Determination of Total Flavonoid Content (TFC)

Total flavonoid content was measured using the modified method adapted from Chang, *et al.*[8] The absorbance was read at wavelength 434 nm. The analysis was done in triplicate. The total flavonoid content was reported as total quercetin equivalent per g sample (mg QE/g).

Statistical Analysis

All the experiments were carried out in triplicate and the data were expressed as a mean ± SD. All analyses were performed using GraphPad Prism version 7 (GraphPad Software, USA).

RESULTS AND DISCUSSION

Pereskia saccharose Griseb. leaves were extracted by maceration using 80% of ethanol. The yield extract obtained were 21.45%. The crude extract then partitioned using n-hexane, ethyl acetate, and n-butanol. The highest percentage yield is from the n-butanol fraction with 14,26%, followed by n-hexane fraction with 8%. The ethyl acetate fraction had the lowest percentage yield (4.22%). Those ethanolic extract and its fraction in n-hexane, ethyl acetate, and n-butanol then examined its ACE inhibitory activity, total phenolic content, and total flavonoid content.

ACE Inhibitory Activity

The ACE inhibitory activity of the ethanolic extract of *Pereskia saccharose* Griseb. leaves were analyzed using ACE Kit-WST (Dojindo, Japan). The assay is based on the detection of 3-hydroxybutyric acid (3HB) derived from 3-hydroxybutyryglycyl-glycyl-glycime (3HB-GGG) by the action of ACE and aminoacylase.[9] Captopril was used as positive control. The ethanolic extract and all fractions were tested for the ACE inhibitory activity at 4.167 ug/mL final concentration. The ethanolic extract showed high percentage inhibition with a value of 50.66% at 4.167 ug/mL final concentration. Among the three fractions, ethyl acetate fraction showed the strongest ACE inhibitory activity at 89.38%, followed by n-butanol (81.83%) and n-hexane (70.45%) (Figure 1).

Ethyl acetate fraction which had the strongest ACE inhibitory activity was further investigated for their effect at a various concentration to obtain the IC_{50} values. The IC_{50} values of the ethanolic extract and ethyl acetate fraction are shown in Table 1. The ethanolic extract gave a high activity with IC_{50} values of 3.448 ug/mL. The ethyl acetate fraction also gave a high activity with IC_{50} values of 1.714×10^{-3} ug/mL. However, the ACE inhibitory activity of captopril as a positive control was more potent than the tested extract and fractions.

Determination of Total Phenolic Content

The determination of total phenolic content was performed using Folin-Ciocalteu method. The total phenolic content was expressed in term of gallic acid equivalent using the standard curve equation $y = 0.1289x + 0.0663$, $R^2 = 0.9975$. The total phenolic content of ethanolic extract of *Pereskia saccharose* Griseb. leaves and its fractions results are showed in Figure 2.

The result showed that the ethanolic extract of *Pereskia saccharose* Griseb. leaves had a high total phenolic content (23.754 ± 0.399 mg GAE/g). Among the three fractions, ethyl acetate fraction contained the highest amount of TPC which is 72.991 ± 0.932 mg GAE/g sample, followed by an n-butanol fraction (59.296 ± 1.06 mg GAE/g) and n-hexane fraction (14.444 ± 0.19 mg GAE/g). The high phenolic content of ethyl acetate fraction may have contributed towards its ACE inhibitory activity.

Determination of Total Flavonoid Content

The determination of total flavonoid content was performed using a colorimetric method with $AlCl_3$ as the chromogenic reagent. The total flavonoid content was expressed in term of quercetin equivalent using the standard curve equation $y = 0.0596x - 0.0339$, $R^2 = 0.9846$. The total flavonoid content of ethanolic extract of *Pereskia saccharose* Griseb. leaves and its fractions results are showed in Figure 3.

The result showed that the ethanolic extract of *Pereskia saccharose* Griseb. leaves had the total flavonoid content of 19.558 ± 1.513 mg QE/g extract. Among the three fractions, ethyl acetate fraction contained the highest amount of TFC which is 61.337 ± 1.612 mg QE/g sample, followed by n-hexane fraction (45.621 ± 0.336 mg QE/g) and n-butanol fraction (27.053 ± 2.002 mg QE/g).

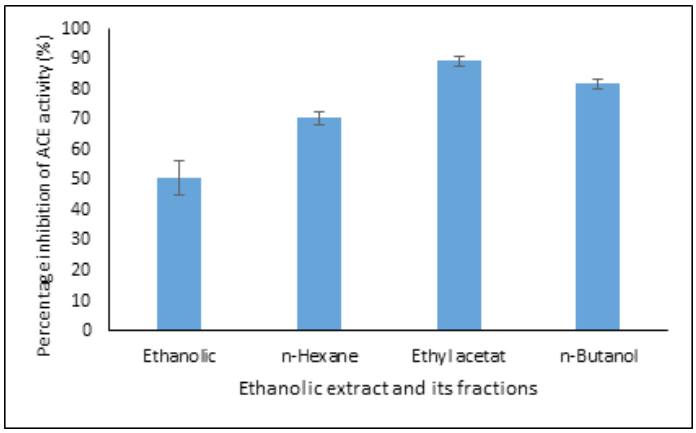

Figure 1: Percentage inhibition of ACE activity by ethanolic extract of *Pereskia saccharose* Griseb. leaves and its fractions at 4.167 ug/mL final concentration.

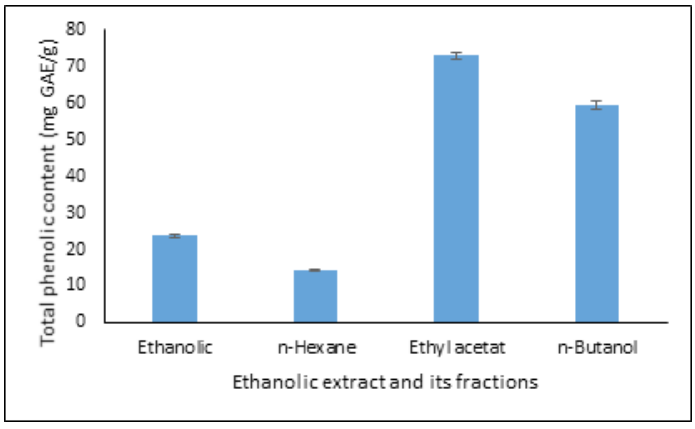

Figure 2: the Total phenolic content of ethanolic extract of *Pereskia saccharose* Griseb. leaves and its fractions.

Table 1: Inhibition of ACE activity by ethanolic extract of Pereskia saccharose Griseb. leaves, ethyl acetate fraction and captopril at various concentration

Samples	IC_{50} values (ug/mL)
Ethanolic extract	3.448
Ethyl acetate fraction	1.714 x 10-3
Captopril (positive control)	1.13 x 10-12

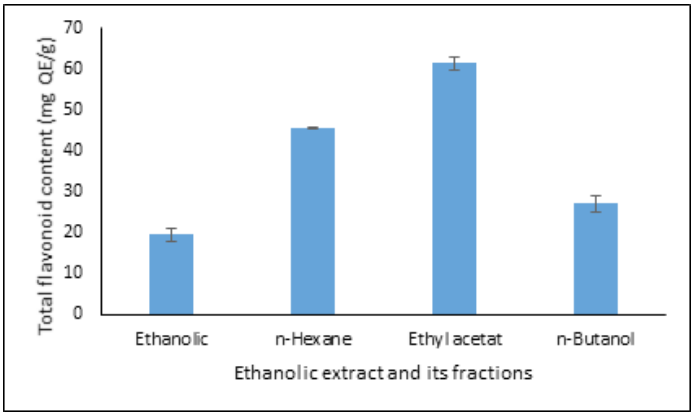

Figure 3: Total flavonoid content of ethanolic extract of *Pereskia saccharose* Griseb. leaves and its fractions.

CONCLUSION

Pereskia saccharose Griseb. leaves can be a good source of ACE inhibitory activity. Further studies need to be carried out to identify the active compounds for development contained in the leaves that lead to structures with the maximum inhibitory activities on ACE activity.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

ABBREVIATION USED

ACE: Angiotensin-converting enzyme; **TPC:** Total phenolic content; **TFC:** Total flavonoid content.

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