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Original Article

Inhibition of Alpha-Glucosidase and Antioxidant Test of Stem Bark Extracts of *Garcinia fruticosa* Lauterb

Nusaibah Zahratunnisa, Berna Elya*, Arikadia Noviani

ABSTRACT

Introduction: Diabetes mellitus (DM) is one of the global health emergencies that characterized by high blood glucose levels (hyperglycemia). Type 2 DM is the most common type in diabetic populations. Inhibition of alpha-glucosidase can ameliorate postprandial hyperglycemia that occurs in patients with type 2 DM. Adding antioxidants to the therapy of DM is intended to reduce complications caused by oxidative stress. Some species of *Garcinia* have been proven to inhibit alpha-glucosidase and have antioxidant activity, but there is no research on *Garcinia fruticosa* Lauterb. Therefore, the aims of this research were to determine the activity of *Garcinia fruticosa* Lauterb. stem bark in inhibiting alpha-glucosidase and as an antioxidant. **Methods:** In this research, the *Garcinia fruticosa* Lauterb. stem bark was dried, grinded, and extracted by multistage maceration using n-hexane, ethyl acetate, and methanol. Inhibition of alpha-glucosidase test has been done *in vitro* on concentrated extracts and measured by microplate reader at 400 nm. The antioxidant test has been done using DPPH scavenging method and was measured by microplate reader at 519 nm. **Results:** Ethyl acetate extract is the most active extract for both test. IC_{50} values for inhibition of alpha-glucosidase test are 20.18 µg/mL that is more active than standard (acarbose) which has IC_{50} value 141.55 µg/mL. Meanwhile, IC_{50} value from an antioxidant test is 8.93 µg/mL that is not more active than standard (quercetin) which has IC_{50} value 2.51 µg/mL. **Conclusion:** Phytochemical screening shows that the ethyl acetate extract contains alkaloids, flavonoids, glycosides, saponins, and tannins.

Key words: Alpha-glucosidase, Antioxidant, DPPH, *Garcinia fruticosa* Lauterb. Stem bark, Phytochemical screening.

Nusaibah Zahratunnisa, Berna Elya*, Arikadia Noviani
Department of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Universitas Indonesia, Kampus Baru UI Depok, 16424, Depok, INDONESIA.

Correspondence

Berna Elya

Faculty of Pharmacy, Universitas Indonesia, Gedung A Rumpun Ilmu Kesehatan Lantai 1, Kampus UI, Depok, Jawa Barat – 16424, INDONESIA.

Phone: +62 21 727 0031

E-mail: berna.elya@gmail.com

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INTRODUCTION

Diabetes mellitus (DM) is one of the leading causes of death and disability in worldwide.[1] DM is characterized by hyperglycemia caused by abnormalities in insulin secretion, insulin action, or both. Prevalence of DM increase annually.[2] Inhibition of alpha-glucosidase enzyme is one of the antidiabetic mechanism that uses in DM therapy, for example, acarbose. This mechanism can inhibit glucose absorption so can prevent hyperglycemia.[3] As a single therapy, acarbose is less effective because it is only 2% absorbed.[4] Chronic hyperglycemia can cause many complications such as damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels.[1] Hyperglycemia has an important contribution in causing complications because of the trigger in free radicals reaction.[5] Adding antioxidants in the DM therapy may prevent oxidative stress that caused by free radicals so that complications can be prevented.[6]

There are many kinds of research on species of *Garcinia* about their activity as alpha-glucosidase inhibitors and antioxidants but there is no research on *Garcinia fruticosa* Lauterb. Ethanolic extract of *G. daedalanthera* Pierre. stem barks showed that the extract can inhibit α-glucosidase with IC_{50} value 3.71 µg/ml.[7] Besides that, methanolic extract of *G. lateriflora* Blume *varJavanica* Boerlleaves showed that the extract has antioxidant activity using DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging method with IC_{50} value 6.18 µg/ml.[8] Based on chemotaxonomic consideration, *Garcinia fruticosa* Lauterb. could be expected to inhibit alpha-glucosidase and has antioxidant activity so could be used as a DM therapy.

MATERIALS AND METHODS

Plant Material

The stem bark of *Garcinia fruticosa* Lauterb. was collected in January 2016 from Bogor, Indonesia and identified by Center for Plant Conservation-Bogor Botanical Garden.

Extraction

The dried stem bark of *Garcinia fruticosa* Lauterb. (780 g) was powdered and extracted consecutively with n-hexane, ethyl acetate, and methanol by cold maceration and then evaporated. On each extract is performed inhibition of alpha-glucosidase test and antioxidant test using DPPH scavenging method.

Inhibition of Alpha-Glucosidase Test

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The inhibition of alpha-glucosidase was determined using adopted method.[9] Five mg (equivalent to 90.3 units alpha-glucosidase enzyme) of alpha-glucosidase (Saccharomyces cerevisiae, Sigma-Aldrich, Germany) was dissolved in 50,0mL of phosphate buffer (pH 6.8) containing 100 mg of bovine serum albumin (Sigma-Aldrich, USA) and then diluted 152 µL in 5,0 mL with phosphate buffer (pH 6.8). The reaction mixture consisting 30 μL of samples at varying concentrations was premixed with 36μLphosphate buffer pH 6.8 and 17 μL of 5 mMp-nitrophenyl-α-D-glucopyranoside (Sigma-Aldrich, Switzerland). After preincubating at 39°C for 5 min, 17 μ Lalpha-glucosidase(0.045 units/mL) was added and incubated at 39°C for 15 minutes. The reaction was terminated by adding100μLNa_2CO_3 200 mM. Inhibition of alpha-glucosidase was determined at 400 nm using microplate reader (Versamax ELISA Microplate Reader, USA) by measuring the quantity of p-nitrophenol released from p-NPG. Acarbose was used as positive control of α-glucosidase inhibitor. The concentration of the extract required to inhibit 50% of α-glucosidase activity under the assay conditions was defined as the IC_{50} value.

Antioxidant Activity Test

The DPPH scavenging method was adopted from Bobo-Garcia *et al.*[10] The preliminary antioxidant test was done using n-hexane extract, ethyl acetate extract, and methanol extract with same concentration (100 µg/mL). The IC_{50} value was determined on the most active extract. The reaction mixture consisting 20 μL of diluted samples at varying concentrations was added to 180 µL of DPPH solution (150 µmol/L) in methanol–water (80:20, v/v) and shaken for 60 seconds in a 96-well microplate. After 40 minutes in the dark at room temperature, the absorbance was measured at 519 nm in the microplate reader of Versamax ELISA Microplate Reader (USA). Quercetin was used as a standard at 1.5–3.5 µg/mL. The % DPPH quenched was calculated using:

$$\%\ \text{DPPH quenched} = \left[1 - \left(\frac{A_{\text{sample}} - A_{\text{blanko}}}{A_{\text{control}} - A_{\text{blank}}}\right)\right] \times 100$$

where A sample is the absorbance at 519 nm of 20 µL of extract or standard with 180 µL DPPH solution after 40min; A blank is an absorbance at 519 nm of 20 µL of water with 180 µL methanol–water (80:20, v/v) after 40 min, and Controls the absorbance at 519 nm of 20 µL of water with 180 µL DPPH solution after 40 min.

Phytochemical Screening

Phytochemical screening was performed to determine alkaloid using Mayer, Dragendorff, and Bouchardartreagents; flavonoid using Shinoda Test; glycoside using Molisch reaction; terpenoids using Liebermann-Burchard reaction; tannin using ferrous (III) chloride and Pb (II) acetate; saponin with honeycomb froth test; and anthraquinone with Borntrager test.

RESULTS AND DISCUSSION

Inhibition of Alpha-Glucosidase Test

Inhibition of alpha-glucosidase test was performed in optimal conditions for the enzyme that have been optimized. The optimal conditions include pH 6.8, temperature 39°C, enzyme concentration 0.045 U/mL, and substrate concentration 5 mM. This test use microplate reader (Versamax ELISA Microplate Reader) at 400 nm. Acarbose is used as a standard. The result shows that acarbose has high IC50 value 141.55 µg/mL. This test was performed on all of the extracts with various concentrations. The results show that IC_{50} value for n-hexane extract is 643.20 µg/mL; IC_{50} value for ethyl acetate extract is 20.18µg/mL; and IC_{50} value for methanol extract is 48.88 µg/mL (Table 1). IC_{50} value ethyl acetate and methanol extract lower than acarbose. That means ethyl acetate and methanol extract is better in inhibiting alpha-glucosidase than acarbose. The ethyl acetate extract is the most active extract in this test because it has the lowest IC_{50} value. This result is related to chemical compounds in the extract that can inhibit alpha-glucosidase synergistically, in contrast to acarbose which is a single compound.

Antioxidant Activity Test

The antioxidant test was performed using DPPH scavenging method by microplate reader (Versamax ELISA Microplate Reader, USA) at 519 nm that was the maximum wavelength of DPPH. Quercetin is used as a standard. The result of the antioxidant test for quercetin showed that quercetin has antioxidant activity with IC_{50} 2.51 µg/mL. Preliminary antioxidant test for extracts was done using n-hexane extract, ethyl acetate extract, and methanol extract with same concentration (100 µg/mL).The n-Hexane extract has percent DPPH quenched 17.53%, ethyl acetate extract has 46,52%, and methanol extract has 35,98% (Table 2). Therefore, the most active extract in having antioxidant activity is ethyl acetate extract because it has the highest percent DPPH quenched. This extract was performed to determine IC_{50} value. The result shows that the ethyl acetate extract has IC_{50} 8.93 µg/mL. IC_{50} value extract is higher than standard (quercetin). In the other words, the extract is not more active than quercetin. However, based on Blois classification the extract is a very strong antioxidant because of the IC_{50} value lower than 50 µg/mL. The strong antioxidant activity is related to phenolic and flavonoid compounds contained in extracts.[11]

Phytochemical Screening

The screening was done on the most active extract in both inhibitions of alpha-glucosidase test and antioxidant activity test that is ethyl acetate extract. The results from phytochemical screening show that the extract contains alkaloids, flavonoids, glycosides, tannins, and saponins (Table 3). Alkaloids are discovered can inhibit the alpha-glucosidase activity competitively and non-competitively.[12] Flavonoids can inhibit alpha-glucosidase activity and have antioxidant activity because of the hydroxyl groups.[13,14] Glycosides also have a role in inhibiting alpha-glucosidase because of the similar structure substrate (contains glucose) so that glycosides can bind to active site.[7] Tannins have a role in inhibiting alpha-glucosidase because those can bind to protein make complexes.[15] The hydroxyl groups in tannins have roles in inhibiting alpha-glucosidase and antioxidant activity.[8,12]

Table 1: Inhibition of alpha-glucosidase results

Sample	IC_{50} (µg/mL)
Acarbose	141.55
n-Hexane Extract	643.20
Ethyl Acetate Extract	20.18
Methanol Extract	48.88

Table 2: Antioxidant activity results

Sample	% DPPH quenched	IC_{50} (µg/mL)
Quercetin	-	2.51
n-Hexane Extract	17.53	-
Ethyl Acetate Extract	46.52	8.93
Methanol Extract	35.98	-

Table 3: Phytochemical screening results

Phytochemical Contents	Results
Alkaloid	+
Flavonoid	+
Glycoside	+
Terpenoid	-
Tannin	+
Saponin	+
Anthraquinone	-

CONCLUSION

Ethyl acetate extract is the most active extract in inhibiting alpha-glucosidase (IC_{50} 20.18 µg/mL) and as antioxidant (IC_{50} 8.93 µg/mL).Phytochemical screening shows that the extract contains alkaloids, flavonoids, glycosides, saponins, and tannins.

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CONFLICT OF INTEREST

None

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ABOUT AUTHORS

Zahratunnisa Nusaibah: Undergraduate Student from Faculty of Pharmacy, University of Indonesia Enrolling Apothecary Program in Faculty of Pharmacy University of Indonesia

Elya: Lecturer, Researcher, and Laboratory of Phytochemistry and Pharmacognosy, Faculty of Pharmacy, Universitas Indonesia

Arikadia Noviani: Lecturer, Researcher, and Laboratory of Phytochemistry and Pharmacognosy, Faculty of Pharmacy, Universitas Indonesia, INDONESIA

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