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Original Article

Fractionation and α-glucosidase Inhibitory Activity of Fractions from *Garcinia hombroniana* Pierre Leaves Extracts

Nita Triadisti*, Rani Sauriasari, Berna Elya*

ABSTRACT

Background: Diabetes mellitus become one of the biggest global health problems of the 21st century. Type 2 diabetes play role for the majority of cases of diabetes worldwide which is characterized by the increase of postprandial blood glucose level. Maintaining postprandial glucose level through inhibition of α-glucosidase is one of the essential strategies in the treatment of diabetes. Inhibitory effect of α-glucosidase was commonly used to identify active compounds potentially to treat diabetes**.** Natural resources have potency as antidiabetic that can be used in diabetes treatment. **Objective**: The objective of the study is to separate active fraction in the crude extract of *Garcinia hombroniana* leaves to facilitate obtaining a pure biologically active compound as the α-glucosidase inhibitor. **Methods**: Fractionation to separate active fraction was performed using column and thin layer chromatography methods while α-glucosidase inhibitory activity assay was performed *in vitro* using spectrophotometric methods at λ 400 nm. **Results**: Ethyl acetate and methanol extract of *G. hombroniana* yielded 14 and 12 fractions, respectively. Two fractions with the higher percent inhibition compared to other factions are fraction 8 from ethyl acetate extract (FEA8) and fraction 3 from methanol extract (FM3). The IC_{50} values of FEA8, FM3 and acarbose are 16.370 *μg*/mL, 59.042 *μg*/mL, and 39.534 *μg*/mL respectively. **Conclusion**: Fraction 8 from ethyl acetate extract of *G. hombroniana* leaves (FEA8) was separated and known in this study as the most bioactive α-glucosidase inhibitor agent compared with another extract, fractions, and acarbose.

Key words: Diabetes mellitus, Fractionation, Column chromatography, Thin layer chromatography, α-glucosidase.

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INTRODUCTION

Diabetes mellitus becomes one of the biggest global health problems of the 21st century. International Diabetes Federation (IDF) analyzed that, by 2040 the global figure will grow to 642 million if nothing is done to respond the growing diabetes epidemic.[1] WHO Global report on diabetes shows that since 1980 community is currently living with diabetes has risen from 180 to 422 million.[2] Type 2 diabetes play role for the majority of cases of diabetes worldwide. Rising phenomenon in diabetes has become a critical concern that drives effort in searching for a new drug from natural resources to face this trend. Diabetes characterized by the increase of postprandial blood glucose level. The management of postprandial blood glucose level important in the treatment of diabetes. The α-glucosidase inhibitor is responsible for the degradation of carbohydrates, it slows down the degradation of carbohydrates by inhibiting the activity of α-glucosidase competitively, so postprandial blood glucose level can be decreased and the postprandial blood glucose level comes under control. α-glucosidase inhibitors can be an alternative first line agent and provide several benefits,[3] it has been recommended by American Association of Clinical Endocrinologists and Third Asia-Pacific Region Diabetes Treatment Guidelines as the first-line of treatment for lowering postprandial hyperglycemia. Their efficacy in lowering HbA1c, and reducing post-meal blood sugars, make them suitable for use in type 2 diabetes.[4,5] α-glucosidase inhibitors effective as monotherapy and polytherapy with other diabetes therapeutic agents.[6]

Natural resources have provided a good source of a wide variety of bioactive compounds from which we can develop new therapeutic agents. Many plants used in the treatment of diabetes, and from the study on various parts of plant, leaves are the most promising part for obtaining active compound.[7,8] Indonesia is a mega-biodiversity country, which has a lot of potential medicinal plants to be developed as an active herbal ingredient or lead compound from plant materials for drug development. α-glucosidase inhibitors that have been used clinically is voglibose, acarbose, and miglitol. Limited choice of α-glucosidase inhibitors has motivated research to get a new α-glucosidase inhibitors agents are expected to have better effectiveness.

α-glucosidase inhibitory activity assay of plants was conducted on *Garcinia fruticosa* Lauterb, *Garcinia rigida* Miq, *Garcinia daedalanthera* Pierre, *Garcinia hombroniana* Pierre, *Garcinia kydia* Roxb, and *Garcinia bancana* Miq and resulting that these extracts

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have α-glucosidase inhibitory activity,[9,10] but further studies to obtain the active fraction that has α-glucosidase inhibition activity has not been done. The aim of the study is to separate active fraction in the crude extract of *G. hombroniana* leaves to facilitate obtaining a pure biologically active compound as the α-glucosidase inhibitor.

MATERIALS AND METHODS

Materials

G. hombroniana Pierre leaves extracts were obtained from Laboratory of Photochemistry and Pharmacognosy, Faculty of Pharmacy, University of Indonesia, α-glucosidase from *S. cerevisiae* (Sigma-Aldrich, Singapore), substrate *p*-nitrophenyl-α-D-glucopyranoside (Sigma-Aldrich, Singapore), Sodium hydroxide, Potassium dihydrogen phosphate (Merck), Sodium carbonate (Merck), Bovine serum albumin (Merck), Acarbose (Sigma-Aldrich, Singapore), Analytical grade acetone, ethyl acetate, chloroform, n-hexane, and methanol, Technical grade n-hexane, ethyl acetate, and methanol have been distilled, Silica gel (70-230 mesh) (Merck), TLC Plate (Silica gel 60 F254) (Merck).

Fractionation of G. hombroniana Leaves Extract

Fractionation extract of *G. hombroniana* (20 g) was performed by column chromatography (diameter 4 cm and height 50cm). Silica gel (70-230 mesh) as the stationary phase prepared by mixing 300 g of silica gel and n-hexane was entered into the column. Combinations of solvents with increasing polarity were used as the mobile phase. The wet packing method was used in preparing the silica gel column. Extracts were prepared in a ceramic mortar by silica gel (70-230 mesh) in acetone and left to dry and then gently layered on top of the column. Elution process was performed with solvent gradient systems in polarity using n-hexane, ethyl acetate, and methanol. Ratios of solvent combinations were sequentially used in the gradient elution; n-hexane: ethyl acetate 100:0, 90:10, 80:20, 70: 30, and so on until comparison 0: 100; and then ethyl acetate: methanol 100:0, 90:10, 80:20, 70: 30, and so on until comparison 0: 100. The eluted fractions were collected in aliquots of 100 ml in a bottle. Fractions are then evaporated and tested using thin layer chromatography.

Analytical Thin Layer Chromatography (TLC) and Pooling of Fractions

Analytical TLC used a plate of silica gel 60 F_{254} (Merck). A plate of silica gel was cut out. With capillary pipe, a spot of the sample was applied on a plate about 1.0 cm from the edge. The plate was entered into a small chamber containing the solvent system and then viewed using UV lamp (Camag) to identify chromatogram pattern. The fraction that has the same chromatogram pattern then merged and tested its activity in inhibition enzyme α-glucosidase. Potential fraction as the α-glucosidase inhibitor was identified with analytical TLC (silica gel 60 F_{254}) (Merck), using spray reagent (1% ethanolic solution of aluminum chloride and 10% methanolic solution of sulphuric acid) and then be observed under UV light.

Inhibition of α-glucosidase Assay

The inhibition of α-glucosidase was assessed using adopted method.[11] A volume of 30 µl of sample, 36 µL of 0.1 M phosphate buffer (pH 6.8) and 17 µL substrate *p*-nitrophenyl-α-D-glucopyranoside at concentration of 4 mM were put in 96 well microplate and then preincubated at 37 °C for 5 min. After preincubating at 37°C for 5 minutes, 17 µL (0.08 units/ml) α-glucosidase was added and incubated at 37°C for 15 minutes to get the complete reaction. The reaction was stopped by adding 100 µL Na_2CO_3 200 mM into each well, and absorbance was measured at 400 nm by a microplate reader (Versamax ELISA Microplate Reader, USA). The IC_{50} value which defined as the concentration of the sample needed to inhibit 50% of α-glucosidase activity in assay conditions, analyzed using GraphPad Prism.

RESULTS

Extracts' test results of α-glucosidase inhibition, show that percent inhibition of n-hexane extract is the lowest, so fractionation performed only on ethyl acetate and methanol extract of *G.hombroniana* (Table 1). Fractionation of ethyl acetate and methanol extract of *G.hombroniana* yielded 14 and 12 fractions, respectively (Table 2). Two fractions with the higher percent inhibition compared to other factions are fraction 8 from ethyl acetate extract (FEA8) and fraction 3 from methanol extract (FM3), their TLC profile is shown in Figure 1 and their percent inhibition in various concentrations shown in Table 3. FEA8 became the most

Table 1: α-glucosidase inhibition activity of extracts (100 µg/mL)

No.	Extract	Percent Inhibition (%) ± SEM
1.	n-Hexane	6.343 % ± 1.624
2.	Ethyl acetate	18.143 % ± 2.095
3.	Methanol	22.677 % ± 1.156

Data are mean ± SEM or % ± SEM for triplicate measurements.

Table 2: Weight, eluent of column and percent inhibition of the fractions (100 µg/mL)

Fraction	Weight (g)	Eluent of column	Percent Inhibition (%) ± SEM
FEA1	0.219	*H* / *E* = 9:1	15.500 % ± 2.562
FEA2	0.284	*H* / *E* = 8:2	22.223 % ± 0.445
FEA3	0.362	*H* / *E* = 8:2	12.090 % ± 3.387
FEA4	1.919	*H* / *E* = 8:2	8.913 % ± 0.673
FEA5	5.098	*H* / *E* = 8:2 – 7:3	21.990 % ± 2.050
FEA6	0.909	*H* / *E* = 7:3	17.930 % ± 1.711
FEA7	0.821	*H* / *E* = 7:3 – 6:4	17.603 % ± 1.375
FEA8	0.734	*H* / *E* = 5:5	71.057 % ± 1.178
FEA9	0.599	*H* / *E* = 4:6 – 3:7	23.577 % ± 1.235
FEA10	2.793	*H* / *E* = 2:8 – E / M = 9:1	26.657 % ± 0.930
FEA11	1.412	E / M = 9:1 – 8:2	25.350 % ± 2.796
FEA12	0.299	E / M = 7:3	23.810 % ± 0.718
FEA13	1.728	E / M = 6:4 – 2:8	20.403 % ± 0.678
FEA14	1.424	E / M = 2:8 – 0:10	13.587 % ± 1.060
FM1	0.074	*H* / *E* = 8:2	23.953 % ± 0.479
FM2	0.873	*H* / *E* = 7:3 – 2:8	15.173 % ± 0.415
FM3	0.213	*H* / *E* = 2:8 – E / M = 9:1	30.263 % ± 0.991
FM4	0.607	E / M = 9:1 – 8:2	20.300 % ± 1.571
FM5	0.777	E / M = 7:3	17.380 % ± 0.846
FM6	2.246	E / M = 6:4 – 5:5	14.137 % ± 1.354
FM7	1.889	E / M = 5:5 – 3:7	11.633 % ± 1.285
FM8	1.119	E / M = 2:8 – 5:5	18.300 % ± 2.163
FM9	1.661	E / M = 4:6	19.477 % ± 0.529
FM10	1.512	E / M = 0:10	18.887 % ± 1.444
FM11	1.480	E / M = 0:10	9.150 % ± 1.960
FM12	2.205	E / M = 0:10	9.543 % ± 0.719

Description: FEA = fractions from extract ethylacetate, FM = fractions from extract methanol, H = n-Hexane, E = ethyl acetate, M : methanol.

Data are mean ± SEM or % ± SEM for triplicate measurements.

Table 3: Percent inhibition of FEA8 and FM3

No.	Concentration (µg/mL)	Percent Inhibition (%) ± SEM
	FEA8	
1.	1.5	32.087 % ± 3.588
2.	4.5	33.200 % ± 2.166
3.	10.5	47.380 % ± 1.158
4.	56.25	79.290 % ± 1.370
5.	75	82.003 % ± 1.185
	FM3	
1.	1.5	1.233 % ± 0.841
2.	4.5	4.983 % ± 1.228
3.	37.5	36.683 % ± 1.169
4.	56.25	47.840 % ± 1.109
5.	75	60.867 % ± 0.911

Data are mean ± SEM or % ± SEM for triplicate measurements.

Table 4: IC_{50} values of FEA8, FM3, and acarbose

No.	Sample	IC_{50} (µg/mL)
1.	FEA 8	16.370
2.	FM3	59.042
3.	Acarbose	39.534

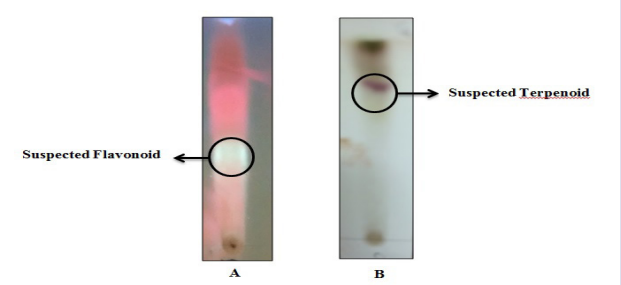

Figure 1: A.TLC Profile of FEA8 B. TLC Profile of FM3

bioactive fraction in α-glucosidase inhibition activity. The IC_{50} values of FEA8, FM3, and acarbose as positive control are presented in Table 4.

DISCUSSION

This study was conducted in order to evaluate α-glucosidase inhibitor potentials of fractions separated from extract of *G.hombroniana* leaves. Antidiabetic properties were determined in terms of the activity to inhibit α-glucosidase. Inhibition of α-glucosidase was exhibited to delay the degradation of carbohydrates. Therefore, α-glucosidase inhibitors have efficacy as therapeutic agents for the management of type 2 diabetes.[12]

Extracts' test results of α-glucosidase inhibition show that percent inhibition of n-hexane extract is the lowest, so fractionation performed only on methanol and ethyl acetate extract of *G.hombroniana* (Table 1). Methanol and ethyl acetate extract of *G.hombroniana* leaves yielded 14 and 12 fractions, respectively using column chromatography. Each fraction produced was evaluated in an *in vitro* α-glucosidase inhibitory studies using microplate reader to facilitate selection of the bioactive fraction.

In vitro α-glucosidase inhibitory studies of fractions demonstrated that all samples had inhibitory activity (Table 2). Fraction 8 from ethyl acetate extract (FEA8) and fraction 3 from methanol extract (FM3) exhibited potency as better α-glucosidase inhibitor compared to other fractions with percent inhibition 71.057 % ± 2.040 and 30.263 % ± 1.716, respectively. Percent inhibition of FEA8 and FM3 in various concentrations shown in Table 3. The highest inhibitory activity of samples was obtained from FEA8 fraction with IC_{50} value 16.370 ug/mL. The IC_{50} value of FEA8 fraction was much smaller than the IC_{50} of acarbose as a positive control (IC_{50} acarbose: 39.534 µg/mL) (Table 4). It shows that FEA8 fraction inhibits the activity of α-glucosidase better than acarbose in a smaller concentration. Assay of α-glucosidase inhibition activity shows that fractions obtained have the higher potency than extract origin, it may because the increase in activity after fractionation may be as a result of separation the active ingredients from the nonactive fractions. The active fraction would usually yield desired biological activity which in this case, is inhibition of α-glucosidase *in vitro* assay. Active fractions, FEA8 was identified by thin layer chromatography using eluent chloroform : ethyl acetate: acetic acid (1:1:0.1), then sprayed with 1% ethanolic solution of aluminium chloride, observed under UV light (366nm), while FM3 was identified by thin layer chromatography using eluent ethyl acetate: methanol (9:1), sprayed with 10% methanolic solution of sulphuric acid and then be heated. Profile TLC of FEA8 show yellow and green fluorescence under UV light (366nm) and FM3 show violet spot (Figure 1). Flavonoids resulting blue, green or yellow fluorescence, which depends on the use of different spray reagents,[13] while terpenoid compounds will form pink to purple or violet after being sprayed with 10% methanolic solution of sulphuric acid and be heated.[14] This result *does not rule out the possibility* that the active fractions contain another phytoconstituents.

The previous studies show that twigs, stem bark, pericarp, and leaves *G.hombroniana* contain alkaloids, flavonoids, phenols, saponins, tannins, xanthones, benzophenones and terpenoids.[15-18] Several studies about α-glucosidase inhibitors obtained from medicinal plants show that terpenes, alkaloids, flavonoids, and phenols have shown α-glucosidase inhibitory activity.[19-21]

This study can be confirmed by further experiments such as flavonoid, phenol, tannin, glycoside, terpenoid, alkaloid isolation from FEA8 and FM3 through preparative HPLC, preparative TLC, column chromatography or recrystallization and α-glucosidase inhibitory activity assay of isolate that have been separated from other compounds.

CONCLUSION

Fraction 8 from the crude ethyl acetate extract of *G.hombroniana* leaves (FEA8) with eluent H/E 5:5 was separated as the most bioactive α-glucosidase inhibitor agent with the highest percentage inhibition compared with another fractions and extracts. The IC_{50} values of FEA8 fraction (16.370 µg/mL) were smaller than the IC_{50} of acarbose as a positive control (39.534 µg/mL), it exhibited higher potency of FEA8 compared to acarbose as positive control. However, further study is needed to isolate bioactive compound in this plant which is responsible for this activity.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ABBREVIATIONS USED

IDF: International Diabetes Federation ; WHO: World Health OOrrganization; TLC: Thin Layer Chromatography ; HPLC: High Performance Liquid Chromatography ; UV: Ultraviolet ; HbA1c: Haemoglobin A1c

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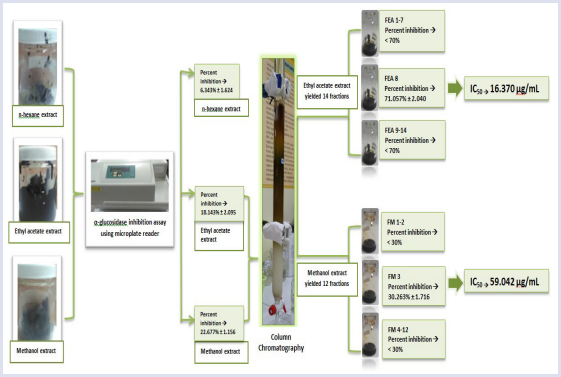
GRAPHICAL ABSTRACT



SUMMARY

- The most active fraction from extract of *G.hombroniana* was separated and tested for α-glucosidase inhibition activity.
- Extracts' test results of α-glucosidase inhibition show that percent inhibition of n-hexane extract of *G.hombroniana* is the lowest, so fractionation performed only on ethyl acetate and methanol extract of *G.hombroniana*.
- Fractionation of ethyl acetate and methanol extract of *G.hombroniana* yielded 14 and 12 fractions, respectively.
- Two fractions with the higher percent inhibition compared to other factions are fraction 8 from ethyl acetate extract (FEA8) and fraction 3 from methanol extract (FM3) with percent inhibition 71.057% ± 2.040 and 30.263 % ± 1.716, respectively.
- The highest inhibitory activity of samples was obtained from FEA 8 fraction with IC_{50} value 16.370 ug/mL was much smaller than the IC_{50} of acarbose as a positive control (IC_{50} acarbose = 39.534 µg/mL)

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