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Research Article

COMPARISON OF INHIBITORY ACTIVITY AGAINST THE α-GLUCOSIDASE ENZYMES IN THE EXTRACTS AND FRACTIONS FROM LEAVES OF THE *GARCINIA KYDIA* ROXBURGH

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ABSTRACT

Objective: *Garcinia kydia* Roxb. is a species of the genus *Garcinia*, is based chemotaxonomic has various bioactive compounds that have been isolated by a variety of pharmacological activities, one of the activities that are being developed that inhibition of α-glucosidase. However, α-glucosidase inhibitory activity in the extracts and fraction from leaves of the *G. kydia* Roxb. has not been reported. In this study, seeks to evaluate of α-glucosidase inhibitory activity against extracts and fractions of potentially.

Methods: The α-glucosidase inhibitory activity test conducted by *in vitro* using the enzymatic reaction is measured of quantity with a microplate reader and identifies the compound from the active fraction with normal-phase thin layer chromatography.

Results: The ethyl acetate and methanol extract have the potential to inhibit the α-glucosidase with the percent inhibition at a concentration of 500 µg/ml of 83% and 59%, respectively. The active fraction of the ethyl acetate extracts (FEA8) with percentage inhibition at concentrations of 100 µg/ml and inhibitory concentration (IC_{50}) values of 80% and 2.79 µg/ml, respectively, and active fraction of the methanol extracts (FMT3) with percent inhibition at concentrations of 100 µg/ml and IC_{50} values of 71% and 8.43 µg/ml, respectively.

Conclusion: *G. kydia* Roxb. evident has the potential to inhibit the α-glucosidase. Flavonoid and phenolic compounds that suspected of acts as α-glucosidase inhibitory activity. Thus, the research will continue the process of isolating the active compound so that it can be developed as natural therapeutic agents in the control of glucose.

Keywords: *Garcinia kydia* Roxburgh, α-glucosidase inhibitory activity, Antidiabetic, *In vitro.*

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INTRODUCTION

The genus *Garcinia* has various bioactive compounds have been isolated which is polyisoprenylated benzophenone derivatives, xanthine derivatives, biflavonoid, and terpenoids with variety of pharmacological activities, namely of anti-inflammatory, antioxidants, antimicrobial, hyperlipidemia agents, and α-glucosidase inhibitors [1].

Therapy of diabetes mellitus with the mechanism of enzyme α-glucosidase inhibition is one of the pharmacological activity of *Garcinia* plants is under development. The α-glucosidase enzyme is a major intestinal enzyme in carbohydrate digestion. Inhibition of these enzymes can delay the process of hydrolysis of carbohydrates, which leads to prevention of excess glucose absorption in the intestine that causes a decrease in blood glucose levels so that the glycemic control can be done better [2].

Diabetes mellitus is a metabolic disease that causes hyperglycemia, due to the lack of the hormone insulin, decreasing the effects of insulin or both [3]. The prevalence of diabetes mellitus is increasing each year. International Diabetes Federation (IDF) reported that the prevalence of diabetes mellitus in 2015 with an age range 20-79 years in Indonesia has ranked 7th with about 10.0 million patients. IDF also stated in 2040 is estimated prevalence will continue to increase, which ranked 6th with 16.2 million people [4]. Hence, the search for active compounds continues to be done.

Some plants of *Garcinia* species have been studied scientifically and are known to have activity as an antidiabetic, among others *Garcinia brevipedicellata* [5], *Garcinia mangostana* [6], *Garcinia nobilis* [7], and *Garcinia hanburyi* [8]. The bioactive compounds that play a role in the α-glucosidase inhibitor from each of these plants that brevipsidones D, prenylated xanthones, xanthones, and polyprenylated xanthones [5-8].

The *Garcinia kydia* Roxb. is one species of the family *Clusiaceae*. In a previous study, the 80% ethanol extracts of leaves has activity against α-glucosidase inhibition with inhibitory concentration (IC_{50}) values of 3.88 mg/ml. Chemical constituents that have been identified, namely, flavonoids, terpenoids, tannins, glycosides, saponins, and anthraquinone [9]. In preliminary testing of secondary metabolites were reported, n-hexane extracts have to contain the steroid/terpenoids, the ethyl acetate extract contains alkaloids, flavonoids, steroids/terpenoids, while the methanol extract contains alkaloids, flavonoids, tannins, anthraquinone, and saponin [10]. However, activity against α-glucosidase inhibition in the extracts and fraction of methanol (FMT), ethyl acetate (FEA) and n-hexane extracts from leaves of *G. kydia* Roxb. has not been reported.

In this study, reported differences in the strength of activity against α-glucosidase inhibition conducted of *in vitro* with measuring the quantity using the microplate reader from various extracts leaves of *G. kydia* Roxb., as well as evaluating the activity of the active fraction of the extract of potentially have α-glucosidase inhibitory activity, which compared to standard acarbose and identify the compound from the active fraction with normal-phase thin layer chromatography (TLC).

METHODS

Collection of plant and extract

The test plants are from the collection of plants Bogor Botanical Gardens in November 2011 and have been identified in the Center for Plant Conservation Indonesian Institute of Sciences, Bogor, Indonesia. The

G. kydia Roxb. leaves extract of n-hexane, ethyl acetate, and methanol obtained from Phytochemistry Research Laboratory of the Faculty of Pharmacy, University of Indonesia.

Fractionation processing

The ethyl acetate and methanol leaves extracts of *G. kydia* Roxb. fractionated by column chromatography using silica gel 60 as stationary phase (230-400 mesh, Merck) [11,12]. The mobile phase is a mixture of the solvent (all the solvents were distilled before being used) with certain ratio, that the obtained gradient eluent system with different polarity. Elution process using solvent mixture of low to high polarity (n-hexane - ethyl acetate - methanol) The fractions have been obtained is then evaporated and the chromatogram profiles identified by the method of TLC using silica gel 60 F_{254} (Merck, 0,25 mm, normal phase) [13,14]. So that the fraction that has the same of chromatogram profile, then combined and conducted of testing the inhibitory activity of the α-glucosidase enzyme.

Determination of the inhibitory activity of α-glucosidase

The inhibitory activity of a-glucosidase was specified using the methods of publications that have been modified [15]. 5 mg of α-glucosidase solid dissolved in a solution of phosphate buffer pH 6.8 (KH_2PO_4 0.2 M and NaOH 0.2 M solution, Merck) which already contains 0.2% bovine serum albumin (Sigma-Aldrich, USA) sufficient to 1.0 ml in low temperature conditions (2-8°C).

The reaction solution containing 30 µl of the sample with different concentrations was added to 36 µl of phosphate buffer pH 6.8 and 17 µl of 4 mM p-nitrophenyl α-D-glucopyranoside (p-NPG) (Sigma-Aldrich, Switzerland) as a substrate. Then, the solution was incubated for 5 minutes at 37°C, and after incubation, the solution was added 17 µl solution of α-glucosidase (Sigma-Aldrich, Germany) 0.08 Units/ml. The solution in incubation return at 37°C for 15 minutes. After that, the reaction is terminated by addition of 100 µl sodium carbonate (Merck) 267 mM.

The inhibitory activity of a-glucosidase was determined at a wavelength of 400 nm with molecular devices spectrophotometer (Microplate reader, VersaMax tunable) by measuring the quantity of product p-nitrophenol resulting from the process hydrolysis of p-NPG [16]. Acarbose (Sigma-Aldrich, USA) can be used as a standard the α-glucosidase inhibitor [17].

Statistical analysis of data

The activity of α-glucosidase inhibitory in each extract and fraction is defined as the Percentage of enzyme inhibition of each extract and fraction was calculated as follows:

$$\%\ \text{Inhibition} = \frac{C\text{-}S}{C} \times 100\%$$

Where C represents the difference between the blank absorbance and control blank absorbance, and S represents the difference between the sample absorbance and control sample absorbance.

The activity of α-glucosidase inhibitory in the active fraction is defined as the difference between the (IC_{50}, concentration that could inhibit the activity of α-glucosidase at 50%) of the active fraction and compared to acarbose as standard, determined by statistically analysis using GraphPad Prism for Windows version 7.0, with the following non-linear regression from equation of three-parameter logistic (GraphPad Software) [18].

Identification of compounds with TLC

The fraction of potentially inhibit α-glucosidase were identified with TLC method [13,19] using stationary phase and mobile phase of silica gel 60 F_{254} and chloroform:ethyl acetate:formic acid (1:1:0.5), respectively. The spots were detected with ultraviolet lamp at 254; by spraying reagent with $AlCl_3$ followed by heating. Thus obtained chromatographic profile.

RESULT AND DISCUSSION

Screening of activities extracts and fractionation

Screening is done by comparing the strength of α-glucosidase inhibitory activity of the n-hexane, ethyl acetate and methanol extract of leaves of *G. kydia* Roxb. at concentrations of 500 and 50 µg/ml which is described by percent inhibition. The results showed that of the three extracts that could potentially have activity in inhibiting the α-glucosidase of an extract of ethyl acetate and methanol, a summary is presented in Table 1.

The ethyl acetate and methanol extract that could potentially, further fractionation by column chromatography method and successively are eluted using the eluent increased of polarity, so that the compound contained in the extract can be separated well by of polarity. The results of fractionated by column chromatography on ethyl acetate and methanol extracts with two stages of identification using TLC led to respectively of 14 fractions and 19 fractions.

α-glucosidase inhibitory potential of extracts, fractions, and the active fraction

The enzyme activity can be demonstrated by the change in color of the substrate, caused by the hydrolysis process of the substrate by the α-glucosidase enzyme to be the color of the product (p-nitrophenol and α-D-glucose). The color change can then be determined quantitatively by measuring the absorbance of p-nitrophenol at a wavelength of 400 nm by microplate reader. If the inhibitor can inhibit the activity of α-glucosidase, then the p-nitrophenol formed will be reduced [20].

Fractions obtained from the FEA and FMT extract, were tested for inhibitory activity of α-glucosidase at a concentration of 100 µg/ml, so that the active fraction from both extracts, namely, fraction 8 (FEA) and 3 (FMT) with the percentage inhibition of 80% and 71%, a summary is shown in Tables 2 and 3.

The examination of α-glucosidase inhibitory activity on of the active fraction with different concentrations of 250, 200, 100, 50, 12.5, and 6,25 µg/ml to obtain the IC_{50} value of 2.79 µg/ml (FEA8) and 8.43 µg/ml (FMT3), the active fraction has IC_{50} values lower than acarbose (39.5 µg/ml). A summary is shown in Tables 4 and 5. This is because the chemical compounds from the fraction is not purified further, so the possibility of a synergistic effect in the inhibition of α-glucosidase.

Identification of compounds with TLC

Based on the identification of ethyl acetate and methanol extracts of leaves *G. kydia* Roxb. using TLC to obtain a chromatogram profile with yellow spots, which indicate the presence of flavonoid compounds [21]. In this study, the identification of compounds against the active FEA

Table 1: Comparison of inhibitory activity against α-glucosidase on the extracts with different solvents

Concentration (µg/ml)	The concentration of the test solution (µg/ml)	% inhibition±SEM		
		n-Hexane extracts	Ethyl acetate extracts	Methanol extracts
500	75	16±2.2	83±0.1	59±2.1
50	7.5	3±3.2	6±3.5	5±2.9

% inhibition values expressed as SEM: Standard error of mean, where n=3

and FMT using the eluent chloroform:ethyl acetate:formic acid (1:1:0.5) obtained some yellow and blue spot indicated of flavonoid compounds, this is because the reaction $AlCl_3$ with the keto group in the C4, C5 and OH group on ortho position of flavonoid compounds to form a complex compound of yellow, chromatogram profiles shown in Fig. 1.

Based on the literature of flavonoid compounds potentially useful in the inhibition of α-glucosidase [22]. Identification of these compounds can be used as data for further research in the isolation of compounds that potentially the inhibition of α-glucosidase (Fig.2).

Table 2: Comparison of inhibitory activity against α-glucosidase of the fractions of ethyl acetate with a concentration of 100 µg/ml (concentration in the test solution 15 µg/ml)

Fraction	% inhibition±SEM
1	7±1.6
2	3±1.9
3	12±5.5
4	9±0.9
5	10±2.1
6	11±5.1
7	75±2.8
8	80±1.7
9	55±7.7
10	38±1.3
11	33±0.9
12	15±2.1
13	12±0.9
14	14±1.9

% inhibition values expressed as SEM: Standard error of mean, where n=3

Table 3: Comparison of inhibitory activity against α-glucosidase of the fractions of methanol at a concentration of 100 µg/ml (concentration in the test solution 15 µg/ml)

Fraction	% inhibition±SEM
1	37±4.6
2	27±2.6
3	71±3.5
4	60±1.9
5	47±1.5
6	40±5.6
7	18±0.3
8	20±0.2
9	36±4.9
10	29±4.6
11	22±1.3
12	18±0.7
13	18±0.7
14	23±0.5
15	23±1.4
16	28±0.9
17	21±3.2
18	27±3.1
19	27±3.8

% inhibition values expressed as SEM: Standar error of mean, where n=3

CONCLUSION

The comparison of α-glucosidase inhibitory activity of leaves extract of *G. kydia* Roxb, obtained the extracts that could potentially inhibit the α-glucosidase, which ethyl acetate and methanol extracts. The comparison the inhibitory between fractions with a concentration of 100 µg/ml for each fraction, thus obtained the active FEA8 and FMT3. The active fraction has stronger activity compared to acarbose, this is indicated by IC_{50} values are generated by the active FEA and FMT is

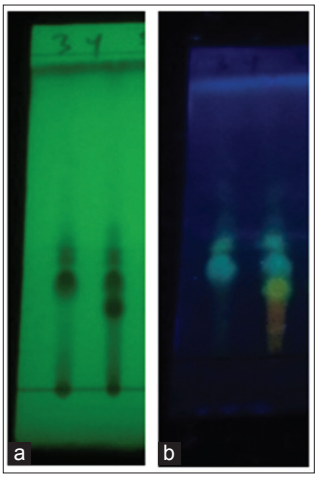

Fig. 1: Chromatography profile of the active fraction of methanol (FMT3 and FMT4) detected by ultraviolet lamp 254 (a) and the spray reagent $AlCl_3$ (b)

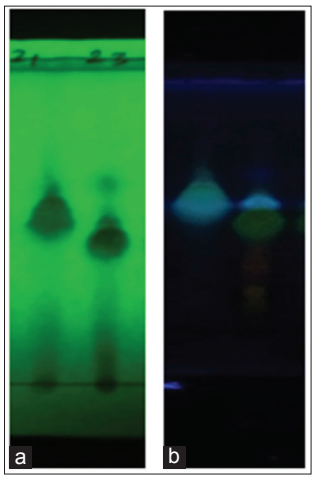

Fig. 2: Chromatography profile of the active fraction of ethyl acetate (FEA8 and FEA9) detected by ultraviolet lamp 254 (a) and the spray reagent $AlCl_3$ (b)

Table 4: α-glucosidase inhibitory potential of the active FEA and FMT

Concentration (µg/ml)	The concentration of the test solution (µg/ml)	FEA8		FMT3	
		% inhibition±SEM	IC_{50} (µg/ml)	% inhibition±SEM	IC_{50} (µg/ml)
250	37.5	92±1.4	2.79	97±0.4	8.43
200	30.0	90±0.5		90±0.7	
100	15.0	80±0.7		78±2.8	
50	7.50	59±1.5		56±3.7	
12.5	1.875	40±4.2		18±3.5	
6.25	0.9375	8±2.2		15±2.4	

% inhibition values expressed as SEM: Standard error of mean, where n=3, IC_{50}: Inhibitory concentration 50%

Table 5: Summary of IC_{50} values the fraction FEA8 and FMT3

IC_{50} values (µg/ml)	
FEA8	2.79
FMT3	8.43
Acarbose (Standard compound)	39.5

IC_{50}: Inhibitory concentration 50%

smaller than acarbose. The active fractions of FEA8 and FMT3 were identified compound, thus acquired chromatogram profile suspected of flavonoid and phenolic compounds. Identification of these compounds can be used as a source of information, so that later can be developed for further research in isolating active compounds that play a role in the inhibition of α-glucosidase, thus acquired natural therapeutic agents that can control blood glucose so as to improve the quality of life of patients with diabetes mellitus.

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