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Alpha-Glucosidase Inhibitory Activity of *Garcinia lateriflora* Blume Leaves

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ABSTRACT

Garcinia lateriflora Blume., that belongs to the genus Garcinia, is known to contain polyphenol compounds that are likely to inhibit alpha-glucosidase in the treatment of diabetes mellitus. Therefore, this study aimed to evaluate and acquire the most biologically active fraction from leaves of *G. lateriflora* in inhibiting alpha-glucosidase. The separation of the active fraction was conducted using column chromatography and identified by thin layer chromatography. Alpha-glucosidase inhibitory activity test carried out in vitro using spectrophotometric method with p-nitrophenyl-α-D-glucopiranoside as the substrate. 17 and 12 fractions were obtained from ethyl acetate and methanol extracts, respectively. Fraction 13 (EA13) of ethyl acetate extract and fraction 10 (Me10) of methanol extract were showed the highest percent inhibition compared with the other fraction with IC_{50} values 8.96 µg/ml and 18.52 µg/ml, respectively. While the IC_{50} value for Acarbose is 39.53 µg/ml. Furthermore, fraction EA13 is the most active fraction in inhibiting alpha-glucosidase compared with the extract, other fractions, and Acarbose.

INTRODUCTION

Diabetes mellitus (DM), as one of metabolic disorder, has become a serious concern in the recent decade. It is associated with a range serious complication that can result in reduced quality of life and premature mortality (WHO, 2016). The prevalence of Diabetes Mellitus was reported to increase from year to year. In 2015 approximately 8.8% of the world population or around 415 million people around the world were reportedly suffering from diabetes. This number is expected to increase to approximately 10.4% in 2040. In 2015, it was reported that the DM has been the cause of death for 5 million people worldwide. The country with the highest population of diabetics are in China, India, USA, Brazil, Mexico, and Indonesia (IDF, 2015). Diabetes mellitus can be characterized by postprandial hyperglycemia, which then can stimulate insulin secretion. Postprandial hyperglycemia can lead to the development of type 2 diabetes and its complications, such as cardiovascular diseases (He *et al*., 2015). One of the therapeutic approaches that can be used to reduce postprandial hyperglycemia is by inhibiting the enzyme which hydrolysis carbohydrates, particularly alpha-glucosidase enzymes in the digestive tract and prevent the absorption of glucose (Derosa and Maffioli, 2012). Inhibition of alpha-glucosidase enzyme can effectively reduce the digestion of complex carbohydrates and its absorption, so that postprandial glucose levels in pre-diabetic patients can be reduced and help to prevent the development of type 2 DM (He *et al*., 2012). Therefore, the mechanism of alpha-glucosidase inhibitor can be one of the potential approaches to find a new agent of drug in the treatment of diabetes mellitus, particularly type 2 DM.

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Plant have long been becoming one of the potential sources of drugs, particularly to treat diabetes mellitus by inhibiting alpha-glucosidase enzyme (Elya *et al*., 2012a; Mun'im *et al*., 2013; Yin *et al.*, 2014). *Garcinia lateriflora* Blume., which belongs to genus Garcinia and family Clusiaceae, is one of the native plants found in Indonesia and mainly used as a folk medicine and foodstuffs by Indonesian native people (Satyanti and Cahyaningsih, 2013).

It has been reported that phenolic compound, such as xanthone and flavonoid, from the extract of *G. lateriflora* stem bark display proteasome-inhibitory activity and, has cytotoxic activity against HT-29 human colon cancer (Ren *et al*., 2010) and P388 cancer cell line (Kosela *et al*., 1999). Another study proved that methanol leaves extract of *G. lateriflora* showed high antioxidant scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (Elya *et al*., 2012c). There are many kinds of research that have been investigated in the inhibitory activities of species from genus Garcinia, but none on *Garcinia lateriflora* Blume. Xanthone from *G. mangostana* (Ryu *et al*., 2011) and depsidone from *G. brevipedicellata* (Ngoupayo *et al*., 2008) had shown alpha-glucosidase inhibitory activity. Leaves extract of G. bancana Miq, G. daedalanthera Pierre, G. kydia Roxb, G. hombroniana, and G. rigida, which were collected from Indonesia, showed moderate to high inhibitory activity of alpha-glucosidase with IC_{50} values 2.33 – 24.48 µg/ml (Elya *et al*., 2012).

Based on chemotaxonomic approach, leaves extract of *G. lateriflora* Blume could be expected to have activity on inhibiting alpha-glucosidase and could be used as one of choices therapy for treatment of diabetes mellitus and prevent the development of its complication.

MATERIAL AND METHODS

Plant materials

The leaves of *Garcinia lateriflora* Blume were collected from the Bogor Botanical Garden, Indonesia. It was identified by The Center for Plant Conservation from Bogor Botanical Garden.

Extraction and Fractionation

Simplisia powder (5 kg) of dried leaves was macerated, successively, with three different distilled solvent, n-hexane, ethyl acetate, and methanol, for 24 h at 25°C. Each extraction was performed three times. Extracts were evaporated by a rotary evaporator to yield n-hexane, ethyl acetate, and methanol crude extracts (Zahratunnisa *et al*., 2017).

Each extract was then tested for the inhibition of alpha-glucosidase. Extract with highest alpha-glucosidase inhibitory activity was further separated. As much as 20 g of ethyl acetate and methanol extracts was fractionated based on previous method (Elya *et al*., 2012c) over column chromatography (4.5 x 40 cm) with stationary phase silica gel 60 and eluted with a step gradient polarity solvent of n-Hexane – Ethyl acetate – Methanol as mobile phase to give 239 and 228 initial fractions. The fraction which had same TLC profile was collected into one fraction to obtain a number of fractions from the extracts. The collected fraction was then tested for the alpha-glucosidase inhibitory activity.

Determination and Identification of Chromatography Profile

Chromatography profile was conducted using TLC silica gel 60 F_{254} plates (Merck, Germany) and determined their profile with UV lamp at 254 and 366 nm (Ahmad *et al*., 2017). The spot was identified by $AlCl_3$ and 10% H_2SO_4 spray reagent (Ahmad *et al*., 2016; Yagi *et al*., 2012).

Inhibition of Alpha-Glucosidase Assay

Inhibition of alpha-glucosidase assay was done by following a previously published method with slight modification (Zahratunnisa *et al*., 2017). 30 µl samples with various concentrations (5 to 500 µg/ml) were put in 96 microplate wells with 36 µl of phosphate buffer pH 6.8 and 17 µl of 4 mM substrate p-nitrofenil-α-D-glucopiranoside (p-NPG) (Sigma-Aldrich, Switzerland).

The mixture then incubated for 5 minutes at 37°C. 17 µl of α-glucosidase (Saccharomyces cerevisiae, Sigma-Aldrich-Germany) at concentration 0.8 unit/L were added to the mixture and incubated for an additional 15 minutes for completing the reduction process of the substrate by the enzyme. The reaction stopped by adding 100 µl of 267 mM Na_2CO_3 solution. A microplate reader (Versamax ELISA Microplate Reader, USA) was used to measure the absorbance of the solution at 400 nm. Each test was repeated three times. Acarbose was demonstrated as a positive control.

A solution system contains substrate and enzyme, without extract was used as blank and solution system without enzyme was used as a control. The percent inhibition of the enzyme by samples were performed by following formula:

Inhibition (%) = [(blank absorption – sample absorption) / blank absorption] x 100.

The fraction with the highest percent inhibition was further calculated in the IC_{50} value. The IC_{50} value showed the concentration of extracts or fractions required to inhibit 50% of α-glucosidase enzyme activity. The IC_{50} value was calculated using GraphPad Prism 7.0 software.

RESULT AND DISCUSSION

Assay for Inhibition of Alpha-Glucosidase of *G. lateriflora* Extract

The inhibitory activity of methanol, ethyl acetate, and n-hexane extracts of *Garcinia lateriflora* Blume. leaves against yeast α-glucosidase enzyme were evaluated. As shown in Table 1, the methanol extract is the most active extract by exhibit the lowest IC_{50} value, followed by ethyl acetate and n-hexane extract, which is 31.27 µg/ml, 34.79 µg/ml, and 92.33 µg/ml, respectively.

Table 1: Percent inhibition and IC_{50} values from extract of *Garcinia lateriflora* Blume. Leaves.

Extract	Concentration (µg/ml)						
	3.18	**7.95**	**15.9**	**23.85**	**39.75**	**79.5**	**IC_{50}**
n-hexane	0.19 ± 0.2	0.73 ± 0.4	2.04 ± 1.1	4.18 ± 1.1	15.35 ± 2.3	41.93 ± 3.1	92.33
EtOAc	3.75 ± 0.1	9.57 ± 0.5	16.23 ± 1.0	27.38 ± 0.6	50.62 ± 1.0	72.66 ± 0.7	34.79
MeOH	0.62 ± 0.1	4.06 ± 0.6	17.77 ± 2.8	32.80 ± 1.3	63.18 ± 0.7	87.96 ± 1.7	31.27
Acarbose	-	-	-	-	-	-	39.53

Percent inhibition was performed by mean ± SEM.

Table 2: Percent Inhibition of fractions from Ethyl Acetate Extract.

Fraction	Percent Inhibition (%)*
EA1 (n-hexane : EtOAc)	15.65 ± 1.0
EA2 (n-hexane : EtOAc)	11.52 ± 1.6
EA3 (n-hexane : EtOAc)	8.39 ± 1.9
EA4 (n-hexane : EtOAc)	4.00 ± 2.0
EA5 (n-hexane : EtOAc)	11.00 ± 2.0
EA6 (n-hexane : EtOAc)	5.95 ± 2.6
EA7 (n-hexane : EtOAc)	15.91 ± 0.7
EA8 (n-hexane : EtOAc)	27.51 ± 5.0
EA9 (n-hexane : EtOAc)	24.77 ± 3.0
EA10 (n-hexane : EtOAc)	32.51 ± 1.41
EA11 (n-hexane : EtOAc)	25.34 ± 1.9
EA12 (n-hexane : EtOAc)	52.28 ± 1.7
EA13 (n-hexane : EtOAc)	64.23 ± 0.9
EA14 (n-hexane : EtOAc)	26.47 ± 2.62
EA15 (EtOAc : MeOH)	18.69 ± 2.5
EA16 (EtOAc : MeOH)	10.95 ± 1.4
EA17 (EtOAc : MeOH)	23.29 ± 2.8
IC_{50} Value (µg/ml)	
Acarbose	39.53
EA13	8.96

*****The percent inhibition (%) was performed in mean ± SEM at consentration 15.9 µg/ml.

Table 3: Percent Inhibition of Fractions from Methanol Extract.

Fraction	PercentInhibition (%)*
Me1 (n-hexane : EtOAc)	8.08 ± 2.3
Me2 (n-hexane : EtOAc)	5.30 ± 1.4
Me3 (n-hexane : EtOAc)	49.49 ± 1.8
Me4 (n-hexane : EtOAc)	26.68 ± 0.5
Me5 (n-hexane : EtOAc)	15.48 ± 2.3
Me6 (n-hexane : EtOAc)	2.78 ± 0.5
Me7 (n-hexane : EtOAc)	12.15 ± 2.8
Me8 (n-hexane : EtOAc)	35.91 ± 1.4
Me9 (n-hexane : EtOAc)	34.89 ± 1.66
Me10 (n-hexane : EtOAc)	58.32 ± 1.6
Me11 (n-hexane : EtOAc)	0.61 ± 0.04
Me12 (n-hexane : EtOAc)	22.61 ± 1.7
IC_{50} Value (µg/ml)	
Acarbose	39.53
Me10	18.52

*The percent inhibition (%) was performed in mean ± SEM at consentration 15.9 µg/ml.

Fractionation and Inhibition of Alpha-Glucosidase Assay of Fractions

Fractionation of ethyl acetate and methanol extracts yielded seventeen and twelve fractions, respectively. Preliminary inhibition of α-glucosidase test was done using 17 fractions of ethyl acetate and 12 fractions of methanol extract with the same concentration (15.9 µg/ml). The results can be shown in Table 2 and Table 3. Fraction EA13 from ethyl acetate extract has percent inhibition 64.23%, which is the highest percent inhibition among others. While, fraction Me10 of methanol extract, with percent inhibition 58.32% have the highest percent inhibition. Therefore, fraction EA13 and Me10 were the most active fraction from each extract. Both most active fraction, EA13, and Me10, was performed to determine IC_{50} value. Fraction EA13 and Me10 has IC_{50} values 8.96 µg/ml and 18.52 µg/ml respectively.

Identification of Fraction

The presence of phenolic compound on fraction EA13 was conducted by $AlCl_3$ spray reagent on a TLC plate with compositions of mobile phase were chloroform: acetone: formic

acid = 4:1: 0.2. It was showing positive reaction by give some spot with a yellowish color and exhibited changing in color at UV lamp 365 nm after spraying by $AlCl_3$. The result can be shown in Fig. 1.

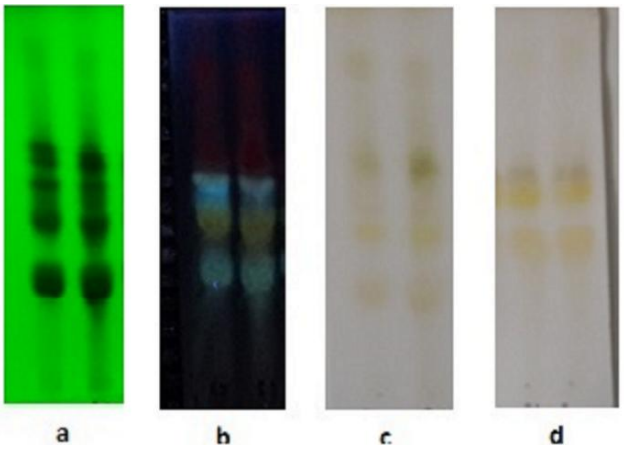

Fig 1: The TLC profile of fraction EA13.
(a) UV lamp 254 nm, (b) UV lamp 366 after sprayed by $AlCl_3$ spray reagent nm, (c) Spot visualized by $AlCl_3$ spray reagent, (d) 10% H_2SO_4.

DISCUSSION

The present research is aimed to evaluate the extracts and fractions from *Garcinia lateriflora* Blume leaves as an alpha-glucosidase inhibitor and obtained the most active fraction. Antidiabetic activities were assessed in terms of the ability to inhibit alpha-glucosidase, an intestinal carbohydrate-digesting enzyme. Alpha-glucosidase inhibitors play a significant role as chemotherapeutic agents in the treatment of type 2 DM by delaying carbohydrate absorption and improve postprandial hyperglycemia (Sugihara *et al*., 2014; Wheni and Tachibana, 2016). However, the present clinical use of alpha-glucosidase in the treatment of type 2 DM, such as Acarbose, miglitol, or voglibose, is lack specificity in their action and cause several side effects. So that, the new alpha-glucosidase inhibitor needed to develop (He *et al*., 2015).

The inhibitory activity of extracts and fractions from the leaves of *G. lateriflora* was investigated against α-glucosidase from *S. cerevisiae* with p-NPG as the substrate. The yellow color was produced from the enzyme's degradation product, p-nitrophenol, and measured using a microplate reader.

Methanol and ethyl acetate extract from leaves of *G. lateriflora* showed moderate inhibition against alpha-glucosidase. These IC_{50} values of methanol extract and ethyl acetate extract were lower than of the positive standard, Acarbose. So that, both of the extracts of *G. lateriflora* leaves are more effective than Acarbose. Methanol extract, which has the highest IC_{50} value, did not show a significant activity at low test concentrations of 3.18 and 7.95 µg/ml, but exhibited higher percent inhibition at high test concentration (15.9; 23.85; 39.75; and 79.5 µg/ml), compared than ethyl acetate extract. This may be due to the ethyl acetate extract contained compounds which can inhibit the alpha-glucosidase enzyme at low concentrations, whereas compounds in the methanol extract, started working synergistically at higher concentrations. The earlier investigation of phytochemistry contents of *G. lateriflora* extracts reported that ethyl acetate extract contains flavonoids, alkaloids, anthraquinone, glycoside and tannins and methanol extract contains alkaloids, saponin, tannins, glycoside, flavonoids and anthraquinones, while n-hexane extract contained steroids/terpenoids (Elya *et al*., 2012b). In terms of this condition, so, both of ethyl acetate and methanol extracts were then purified by column chromatography for further investigate the inhibitory activities of alpha-glucosidase. The percent inhibition of each fraction was indicating that not all of the fractions provided good inhibition of α-glucosidase. This may depend on the difference in the content of active compounds at each fraction. Fraction EA13, which is the most active fraction, was then identified with $AlCl_3$ spray reagent for preliminary phytochemical screening and reacted positively.

This indicates the existence of phenolic compounds (Yagi *et al*., 2012). Phenolic compounds, such as phenolic acid, biphenyls, and flavonoid, or proanthocyanidins have been known can reduce a blood glucose level through its mechanism by inhibiting the carbohydrate digestive enzyme, particularly on alpha-glucosidase (Etxeberria *et al*., 2012). Other research showed that substitution of hydroxyl groups on flavonoid could decrease the inhibitory activity of alpha-glucosidase, therefore increasing the number of free phenolic groups will increase the ability to inhibits alpha-glucosidase (Moradi-Afrapoli *et al*., 2012). The alpha-glucosidase inhibitory activity has been proven in vitro against yeast α-glucosidase or mammalian intestinal α-glucosidase enzyme (maltase, sucrose, and isomaltase) and showed high inhibitory activity in many research (Etxeberria *et al*. 2012; Yao *et al*. 2012; Yin *et al*. 2014). In contrast with the promising inhibitory activity of phenolic compound in vitro (Sivasothy *et al*., 2016), another study showed that no significant inhibition of phenolic compound against rats α-glucosidase enzyme in vivo (Zhang *et al*., 2014).

This research is a preliminary study in order to find active compounds with alpha-glucosidase inhibitory activity. Therefore, further research is needed to isolate the pure lead compound that active biologically as alpha-glucosidase inhibitor from the leaves extract of *G. lateriflora* Blume.

CONCLUSION

In conclusion, we investigated the inhibitory activities of extracts and the separated fractions which performed by column chromatography from *G. lateriflora* Blume. leaves against yeast α-glucosidase. Fraction EA13 of ethyl acetate extract showed promising results in inhibiting α-glucosidase with high IC_{50} value (8.96 µg/ml) under our test condition. Preliminary phytochemical screening with $AlCl_3$ spray reagent showed that the fraction consists of polyphenol compounds. Therefore, we suggest further purification, isolation, and characterization of the lead compound responsible for the inhibitory activity of alpha-glucosidase from the leaves of *G. lateriflora* Blume.

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