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Kinetics of α -glucosidase inhibitory activity and phytochemical analysis of *Piper crocatum* Ruiz & Pav. leaves ethanol extract

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ABSTRACT

α-Glucosidase is an enzymes group that playing essential roles in the digestion of polysaccharide. Inhibitor of a-glucosidase can decrease polysaccharide digestion rate and therefore plays a significant function in preventing the development of diabetes (type 2). *Piper crocatum* Ruiz & Pav. is an essential herb applied traditionally in Indonesia to treat diabetes mellitus. This work evaluated the a-glucosidase inhibitory activity of *P. crocatum* leaves ethanol extract. Phytochemical component of the extract was also analyzed. The α-glucosidase inhibitory activity of the *P. crocatum* leaves ethanol extract was examined by reacting its different concentrations with α-glucosidase and *p*-nitrophenyl glucopyranoside. Kinetics of the α-glucosidase inhibition was determined using a Lineweaver-Burke plot. Phytochemical in the extract was determined using GC-MS. Ethanol extract of *P. crocatum* leaves exhibited moderate α-glucosidase inhibitory activity compared with acarbose. Phytochemical analyses showed the presence of stilbene, linolenic acid, phenol, phytosteroid, and α-tocopherol. The competitive action of *P. crocatum* leaves ethanol extract is due to its inhibitory effects on α-glucosidase. The stilbene and phenol compounds indicated responsible for anti-diabetic activity from *P. crocatum* leaves ethanol extract.

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INTRODUCTION

Diabetes mellitus (DM) is a syndrome metabolite disease with significant mortality and characterized by a high level of blood glucose (hyperglycemia) and disfunction in insulin secretion and/or insulin action (Wilkinson *et al.*, 2019). In 2019, the DM prevalence in the world was an estimated 463 million (Saeedi *et al.*, 2019). It is also predicted that DM prevalence will reach 578 and 700 million in 2030 and 2045, respectively (Saeedi *et al.*, 2019). Generally, the three DM types are type I diabetes with insulin deficiency, type II diabetes with insulin resistance, and gestational diabetes (Zhang *et al.*, 2019). Hyperglycemia condition will produce diabetic complications, such as cardiovascular

diseases (Khouja *et al.*, 2019), chronic kidney disease (Brugnara *et al.*, 2018), blurred vision (Sayin *et al.*, 2015), nephropathy (Elsayed *et al.*, 2019), anxiety and depression (Khalighi *et al.*, 2019), and retinopathy (Gargiulo *et al.*, 2004). Therefore, prevent the occurrence of hyperglycemia condition is an alternative strategy inpatient diabetes management.

The inhibition of the a-glucosidase enzyme (EC 3.2.1.20) is commonly applied to cure diabetes through a mechanism by reducing polysaccharides hydrolysis (digestion) so that glucose absorption is low and consequently does not occur hyperglycemia (Sangeetha and Devi, 2018). Acarbose is a commercial drug of DM which able to inhibit a-glucosidase. Still, it has several more side effects, such as the liver and abdominal illnesses, diarrhoea, and flatulence (Godbout and Chiasson, 2007). Thus, the find of novelty a-glucosidase inhibitor with lest side effect is of enormous significance for diabetes management. Recently, several reports have shown that the medicinal plant as a source of a-glucosidase inhibitor (Nurcholis *et al.*, 2018; Magaji *et al.*, 2020; Trinh *et al.*, 2020). *Piper crocatum* Ruiz & Pav. is a medicinal plant that belongs to the family Piperaceae and traditionally used to treat diabetes mellitus in Indonesia (Safithri and Fahma, 2008). As the reported literature shows, no articles about the influence of different concentrations of *P. crocatum* ethanol extract leaves on a-glucosidase inhibitory, and its phytochemical identification has been presented. This property was evaluated in this study. Thus, the objective of this work is to investigate the mechanism of a-glucosidase inhibitory activity and phytochemical analysis from *P. crocatum* ethanol extract leaves.

MATERIALS AND METHODS

Reagents and chemicals

Acarbose and p-nitrophenyl-a-D-glucopyranoside were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

Plant material and extraction

The *P. crocatum* was collected from Bogor, West Java, Indonesia, and identified by Dr Mega Safithri. The extraction used the maceration method at room temperature. Briefly, 25 g of *P. crocatum* fresh leaves were macerated using 70% ethanol (100 ml). After 2x24 h, the solution filtered and then evaporated at 50 °C and finally dried using freeze dryer at -50 °C and 8 mBar.

a-Glucosidase inhibition assay

The a-glucosidase inhibition of the sample extract and acarbose were measured according to the assay described by (Dubey *et al.*, 2017), with modifications. The α-glucosidase (0.5 mg) dissolved in 0.1 M phosphate buffer (pH 7.0) containing bovine serum albumin (100 mg). Before used, the enzyme solution was diluted 25 times, with 0.1 M phosphate buffer pH 7.0. The sample (20 ml) at various concentrations (0.1% to 1.0%) in DMSO was mixed with 980 ml buffer phosphate pH 7.0 (0.1 M) and 500 ml *p*-nitrophenyl-α-D-glucopyranoside (20 mM). After incubated for 5 min at 37°C, the mixture was added enzyme solution (500 ml) and incubated again for 15 min at 37°C. Finally, the reaction was terminated with 2 ml Na_2CO_3 solution (200 mM), and absorbance at 400 nm was measured with UV-Vis spectrophotometer. The blank was used DMSO with no extract. The a-glucosidase inhibition of the sample extract was calculated as follows:

$$\text{inhibition } (\%) = \frac{(\text{A}_{\text{control}} - \text{A}_{\text{sample}})}{\text{A}_{\text{control}}} \times 100$$

where $A_{control}$ is an absorbance without extract; A_{sample} is an absorbance with the extract.

The kinetic of a-glucosidase inhibition

The mechanism of kinetic inhibition of the sample, in the presence or absence, on a-glucosidase was determined by using assay method in Section a-glucosidase inhibition assay with serial concentration *p*-nitrophenyl-α-D-glucopyranoside (5 – 25 mM) for 15 min at 37°C. Reactions were stopped, and absorption was measured and plotted by the Libewaver-Burk curve.

GC-MS analysis

Phytochemical analysis of *P. crocatum* ethanol extract leaves was performed using the GC-MS instrument. GC-MS equipped with capillary column HP-5 (Agilent 19091J-433; 0:25 mm x 30 m x 0.25 μm; 5% diphenyl, 95% dimethylpolysiloxane). The solution sample (1 ml) injected and used a flow rate of 1 ml/min—the temperature and pressure used of 300°C and 10.47 psi, respectively. Helium gas used as the eluent. Mass parameter detection with masses 50 – 800 (MS quad temperature of 150-200 °C and MS source 250-300 °C) were used to detection of compounds. The identification of mass-spectrum GC-MS was evaluated using the database of NIST.

RESULTS AND DISCUSSION

a-Glucosidase inhibitory activity of *P. crocatum* ethanol extract leaves (PCE) are illustrated in Figure 1. The concentration PCE of 0.1% to 1% have significantly lower inhibition on a-glucosidase activity compared to 1% acarbose (80.97%) at $p < 0.05$.

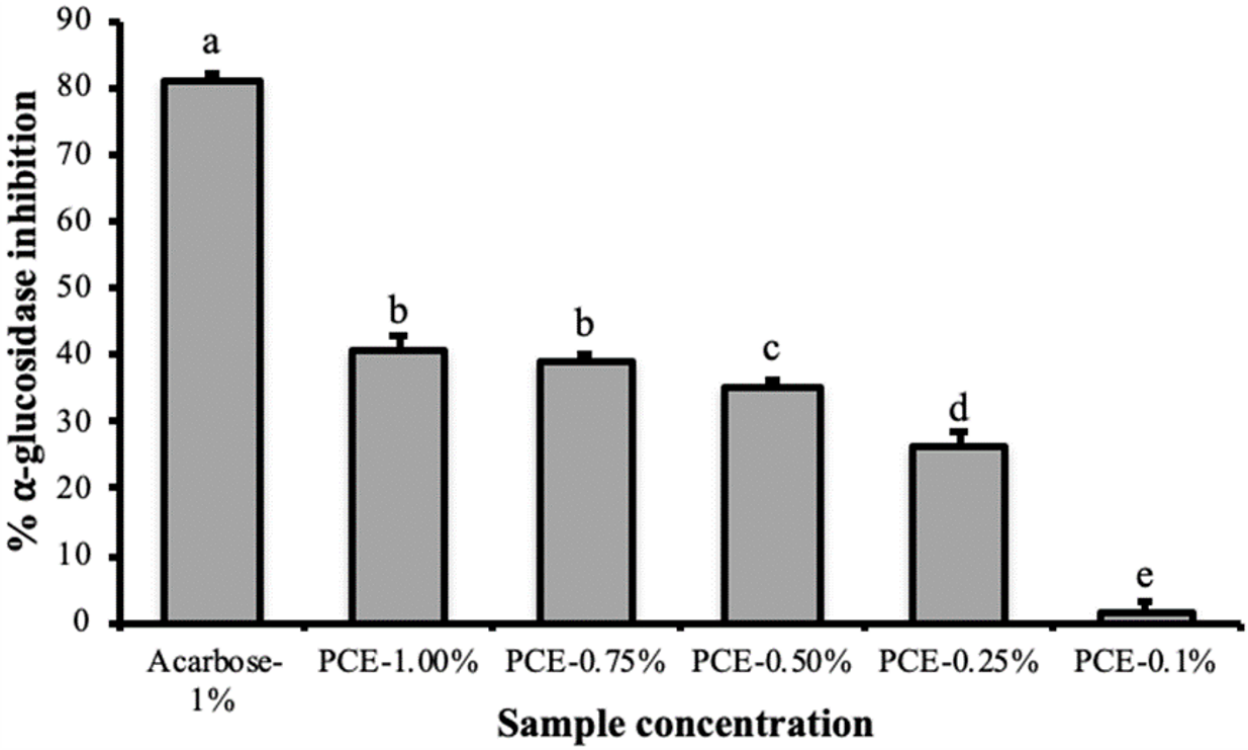

Figure 1: a-glucosidase inhibition activity of *Piper crocatum* ethanol extract (PCE) and acarbose at different concentrations. Different letter on the top of the bar chart showed a significant difference at $p < 0.05$

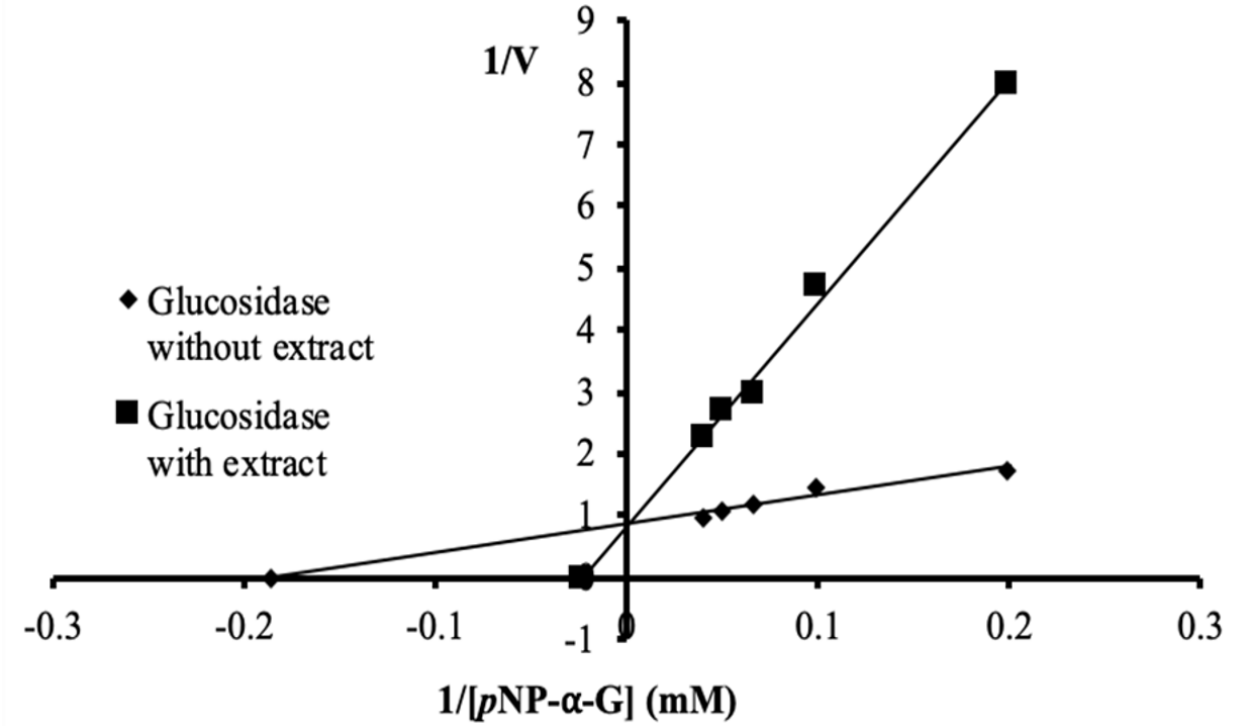

Figure 2: Lineweaver-Burk plots of a-glucosidase inhibition at different concentrations of *p*-nitrophenyl-α-D-glucopyranoside (PnP-α-G) by *Piper crocatum* ethanol extract

Table 1: Metabolite identified in the *Piper crocatum* ethanol extract by GC-MS

No	Retention Time (min)	Area (%)	Metabolite
1	12.07	6.13	Linolenic acid
2	24.89	12.19	Phenol
3	26.12	4.52	Phytosteroid
4	27.20	44.69	Stilbene
5	36.46	1.65	a-Tocopherol

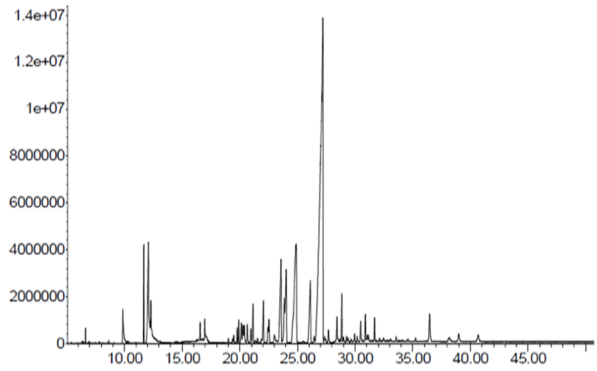

Figure 3: GC-MS chromatogram of *Piper crocatum* ethanol extract

The percentage of inhibition value of PCE ranged from 1.29% to 40.80%. These results indicated that the percentage of a-glucosidase inhibition increased in the rising concentration of PCE.

These data were coherent with the results of previous work on the beverage formula of P.*crocatum* (Safithri and Kurniawati, 2016; Syaefudin *et al.*, 2016)

The enzyme kinetics of the a-glucosidase in the presence of the PCE (inhibitor/extract) was determined from Lineweaver-Burk curve. The Lineweaver-Burk plot is a general technique for evaluation of the enzyme kinetics (Rouzbehan *et al.*, 2017). The inhibition kinetics of PCE on a-glucosidase is shown in Figure 2. The line showed the same in Y-intercept between a-glucosidase with extract (PCE) and without extract. This result indicated that the PCE exhibited a competitive type of a-glucosidase inhibition. This mechanism action of competitive is similarly with acarbose on a-glucosidase inhibition (Prasad *et al.*, 2019). Therefore, the higher concentration of PCE is required to decrease the hyperglycemia condition.

The chromatogram of GC-MS of the PCE presented in Figure 3. The metabolite was identified based on a comparison between NIST library with the mass spectra of the compounds; the five metabolites were identified and characterized (Table 1). The stilbene was the primary compound on PCE (44.69%) followed by phenol (12.19%), linolenic acid (6.13%), phytosteroid (4.52%), and a-tocopherol (1.65%). Among the metabolites, stilbene and phenol were reported to have a-glucosidase inhibitory activity (Zhang *et al.*, 2017; Figueiredo-González *et al.*, 2019).

CONCLUSIONS

We concluded that *P. crocatum* ethanol extract leaves possess of a-glucosidase inhibitory activity with competitive mode for kinetic enzyme mechanism. The metabolite of stilbene and phenol of *P. crocatum* ethanol extract leaves may be a role for its anti-diabetic properties.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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