The in Vitro Anti-Diabetic Activity of Lime Peels (*Citrus Amblycarpa* (HASSK.) OCHSE)

Gempita Cahaya Aulia Tambunan
Prima University of Indonesia

Aparna Dutt
Prima University of Indonesia

Sayra Nadhifa
Prima University of Indonesia

Firdha Amelia
Prima University of Indonesia

Ermi Girsang
Prima University of Indonesia, ermigirsang@unprimdn.ac.id

Abstract

There are various potential natural anti-diabetic drugs; one of them is lime peel or *Citrus amblycarpa*. This study was aimed to explore the anti-diabetic activity and phytochemical content of lime peels. This study was an quasy experimental study that used the post-test only control group design. The lime peels that were collected from the Berastagi fruit market in Medan, North Sumatera were extracted using 70% ethanol by maceration methods. The phytochemical screening identified the presence of phenolic, steroid/triterpenoid, terpenoid, saponin, flavonoid, tannin, and alkaloid. Meanwhile, the anti-diabetic activity of lime peels was evaluate using the α-glucosidase enzyme that was gotten from Saccharomyces cerevisiae by α-glucosidase enzyme inhibition methods. Percent of inhibition was express as Mean ± SD and analyzed by One Way ANOVA, Tukey HSD Post Hoc Test, and followed by linear regression. The result of this study showed that there is a significant difference in percentage inhibition α-glucosidase enzyme in each concentration, and it had an IC50 Value amount of 125.93 ± 9.14 µg/mL. The phytochemical content of the lime peels was flavonoid, phenol, steroid/triterpenoid, and alkaloid. Hence, the lime peel has anti-diabetic activity by inhibition of the α-glucosidase enzyme.

Keywords: Lime peel, α-glucosidase enzyme, anti-diabetic, Citrus amblycarpa

INTRODUCTION

Diabetes Mellitus Type 2 or Insulin Independent Diabetes Mellitus is a chronic disease that requires intense treatment and strategies to reduce risk factors and control blood glucose. Based on the estimation of WHO (World Health Organization), around 171 million people over the world in 2000 suffered from diabetes and will increase and reach 366 million people in 2030. Meanwhile, based on the estimation of ADA (American Diabetic Association), the cost of diabetic care in the United States spends a national funds amount of $US 132 billion in 2002 and will increase to $US 192 billion in 2020 (American Diabetes Association, 2015; World Health Organization, 2016).

The prevalence of diabetes in Indonesia was reported by an annual report

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named Riskesdas in 2013 showed that the prevalence of diabetes was 1.5 percent in Indonesia that was diagnosed by the doctor, and the prevalence varied in several provinces in Indonesia. The top three provinces that had diabetes mellitus people that were diagnosis by the doctor were Yogyakarta (2.6%), Jakarta (2.5%), North Sulawesi (2.4%), and East Kalimantan (2.3%) (Badan Penelitian Dan Pengembangan Kesehatan Kementerian Kesehatan RI, 2013).

There are four types of oral anti-hyperglycemic drugs. These were insulin sensitive sensitizing, insulin secretagogues, glucosidase inhibitors, and incretins. Acarbose is one of the oral anti-hyperglycemic drugs that inhibit the α-glucosidase enzyme in the human body. This drug acts as a competitive inhibitor for α-glucosidase enzyme (Soegondo, 2017).

Glucosidase enzyme is an enzyme that catalyzes the hydrolysis of glycoside bonds in oligosaccharides or glycoconjugates. This enzyme specifically hydrolyzes the glycoside bond in the sugar molecule. There are two types of this enzyme, these were alpha and beta-glucosidase that hydrolyze the glycoside bonds in both alpha and beta form. The alpha glucoside bond is a glucose bond with the hydroxyl group under the plane of the ring, while the beta bond is above the ring plane (Borges de Melo, da Silveira Gomes and Carvalho, 2006; Gordon, 2010).

As one of the oral anti-Hyperglycemic drugs, glucosidase enzyme inhibitor is a competitive inhibitor against the alpha-glucosidase enzyme. This enzyme is found in the brush border of the intestine to hydrolyze oligosaccharide, trisaccharide, and disaccharide. On the other hand, this enzyme is also found in the liver that prevents glycogenolysis for control the blood glucose level in the normal range (Borges de Melo, da Silveira Gomes and Carvalho, 2006; Naquvi *et al.*, 2011).

There are various natural sources that had anti-diabetic activity by inhibition of alpha-glucosidase enzyme with various degrees of inhibition. These are *Gymnrma sylvestre*, *Momordica charantia*, *Trigonella foenum graecum, Pterocarpus marsupium, Murraya koenigii*, and other natural sources that have the inhibition activity. One of these natural sources that have not been explored in the anti-diabetic activity is lime (*Citrus amblycarpa*). Another type of *Citrus'* has anti-diabetic activity through the alpha-glucosidase inhibition or amylase enzyme inhibition. *Citrus amblycarpa*, as part of Citrus, may have the same anti-diabetic activity. The preceding study that was reported by Putra et al. (2018) showed that the ethanol

extract of *Citrus amblycarpa* leaf has several phytochemicals like flavonoid, polyphenol, tannin, and glycoside that was also found in the other type of Citrus that showed the anti-diabetic activity (Putra, Satriawati and Astuti, 2018). Based on the information above, this study was aimed to explore the anti-diabetic activity of the lime (*Citrus amblycarpa*) peel and its phytochemical content.

METHODS

This was an experimental study that used the post-test only control group design. The lime that was used in this study was gotten from the Berastagi fruit market in Medan, North Sumatera. The lime was identified in the Herbarium Laboratory of School of Life Sciences and Technology – Science Program in Bandung Institute of Technology. While overall of this research was conducted in the Aretha Medika Utama Biomolecular and Biomedical Research Center in Bandung.

The lime was washed, and the fruit peel was separated from the fruit pulp. This was dried by a food dehydrator and meshed into Simplicia powder. The simplicia powder was extracted using 70% ethanol for 24 hours by maceration methods. After that, these were filtered, and the residue was re-macerated for two days. Meanwhile, the filtrate of maceration and re-maceration process was collected to be evaporated by a rotary vacuum evaporator at 50^{o}C to form a concentrated form of extracts that were known as ethanol extract of lime peel and seed (Widowati *et al.*, 2014, 2016, 2017; Widowati, Widya Janeva, *et al.*, 2018).

Before the α-glucosidase enzyme inhibition assay was performed, the ethanol extract was performed the phytochemical screening process based on the procedure that was described by Widowati et al. (Widowati *et al.*, 2016, 2017; Widowati, Widya Janeva, *et al.*, 2018). The phytochemical screening identified the presence of phenolic, steroid/triterpenoid, terpenoid, saponin, flavonoid, tannin, and alkaloid.

The α-glucosidase enzyme inhibition assay should be begun by preparing the enzyme solution. The enzyme solution was made by mixing 1 mg of the α-glucosidase enzyme into 100 ml buffer phosphate (pH 7.0) that contain 200 mg of bovine serum albumin prior to being used the solution had to dissolve by 1/50 of buffer phosphate. Furthermore, the amount of 25 µL of enzyme solution was mixed into substrate solution that contains 25 µL p-nitrophenyl α-D-glucopyranoside 20 mM as the substrate, 45 µL buffer phosphate, and 5 µL sample solution (DMSO solution as the control). This mixture was incubated

in the 37°C for 30 minutes. After that, the reaction was terminated by adding 100 µL 0,2 M Na_2CO_3 solution. The absorbance was measured at 400 nm wavelength by spectrophotometry, and the percent of enzym inhibition was determined by the following formulation (Widowati *et al.*, 2011; Gondokesumo, Kusuma and Widowati, 2017; Pujimulyani *et al.*, 2018; Widowati, Wargasetia, *et al.*, 2018):

$$\frac{(C-S)}{C} \text{ X } 100\%$$

C: Absorbance of the control

S: Absorbance of the sample

The percentage of enzyme inhibition was express as Mean ± SD. The percent of enzyme inhibition was analyzed by One Way ANOVA and followed by Tukey HSD Post Hoc Test. Furthermore, the analysis was continued by linear regression to determine the Inhibition Concentration 50 (IC_{50}) of each extract.

RESULT

Identification of Sample

The result of the identification of the sample that was used in this study was showed that it was lime or *Jeruk Sambal* in Indonesia that had scientific name *Citrus amblycarpa* (Hassk.) Ochse.

Kingdom : *Plantae*

Division : *Magnoliophyta*

Class : *Magnoliopsida*

Subclass : *Rosidae*

Ordo : *Sapindales*

Family : *Rutaceae*

Genus : *Citrus*

Species : *Citrus amblycarpa* (Hassk.) Ochse

Synonym : *Citrus limonellus* var. *amblycarpa* Hassk.

Yield of Extract

This study was used lime peels as the sample. The sample was extracted using 70% ethanol by maceration methods. The yield of extraction is shown by the following table.

Table 1 The yield of Ethanol Extract from Lime Peel

Weight of Simplicia Powder (g)	The volume of 70% Ethanol	Duration of Maceration	Volume of Filtrate	Weight of Extract (g)	Yield of Extract
150	500 mL	Three days	350 mL	59.6	39.7

Phytochemical Screening

The result of the phytochemical screening of Ethanol extract from Lime Peels is shown by the following table.

Table 2 The Screening Phytochemical of Lime Peels Extract

Phytochemical	Result
Flavonoid	+
Saponin	-
Phenol	+
Tannin	-
Steroid/Triterpenoid	+
Terpenoid	-
Alkaloid	+

Based on the table above, the ethanol extract of lime peels has some

phytochemicals includes flavonoid, phenol, steroid/triterpenoid, and alkaloid.

α-Glucosidase Enzyme Inhibition Assay

The result of the α-Glucosidase enzyme inhibition assay was shown as a percent of inhibition. Furthermore, the percent of α-Glucosidase enzyme inhibition was compared among each group of concentration, and the result of the comparison was shown by the following table.

Table 3 Comparison of α-Glucosidase enzyme inhibition Activity in each concentration of Ethanol Extract from Lime Peel

Concentration (µg/mL)	Percent of Inhibition (%)
3.13	6.60 ± 0.83^{a}
6.25	12.79 ± 1.16^{ab}
12.50	12.30 ± 1.36^{ab}
25.00	16.33 ± 1.61^{b}
50.00	24.73 ± 0.95^{c}
100.00	45.20 ± 0.55^{d}
200.00	73.05 ± 6.21^{e}

Data were expressed as Mean ± SD. The difference superscript showed significance at P-Value < 0.05 based on the Tukey HSD Post Hoc Test.

Based on the table above, the lower concentration showed a significant difference in percentage inhibition at P-Value < 0.05. However, at the higher concentration, the difference in percentage inhibition was not significant. It means that in higher concentration, the reaction was begun to be saturated. Furthermore, the analysis was continued by linear regression to determine the IC_{50} value, and the result of the analysis was showed by the following table.

Table 4. Analysis of Linear Regression against Percentage Inhibition

Repetition	Equation	R^2	IC_{50} (µg/mL)	IC_{50} (µg/mL)
First repetition	Y = 0.3614x + 7.4823	0.99	117.65	
Second repetition	Y = 0.2982x + 9.5231	0.98	135.74	125.93 ± 9.14
Third repetition	Y = 0.333x + 8.5752	0.99	124.40	
Average	Y = 0.3309x + 8.5269	0.99	125.33	

Based on the table above, the IC50 of ethanol extract from lime peels was 125.95 125.93 ± 9.14 µg/mL. It means that it required 135.07-116.79 µg/mL ethanol extract of lime peels to inhibition 50% of α-Glucosidase.

DISCUSSION

The result of this study showed that the increasing concentration of ethanol extract leads to an increase in the inhibition activity against the α-Glucosidase enzyme. That was shown by the following figure.

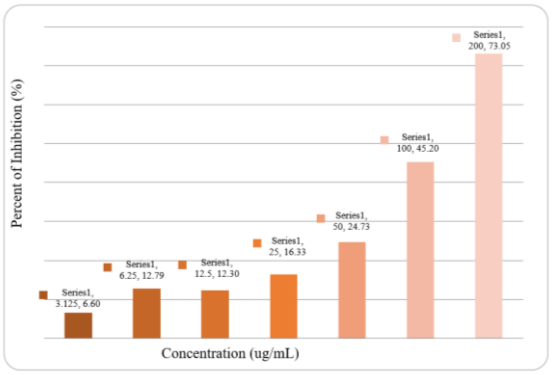

Figure 1. Effect of Various Concentration of Ethanol Extract from Citrus amblycarpa against Inhibition of α-Glucosidase Enzyme

The α-Glucosidase enzyme is usually found in the human intestine to degrade carbohydrates. This enzyme hydrolyzed the α-glycoside bone on the oligosaccharide and produced α-D-glycoside or glucose. The method that used to evaluation of the inhibition activity was based on the ability of sample for inhibiting the reaction of the enzyme against p-nitrophenyl-α-D-glucopyranoside (p-NPG), and this reaction would produce α-D-glucose and p-nitrophenyl that had a yellow color. The reaction was shown by the following figure (Widowati *et al.*, 2011; Gondokesumo, Kusuma and Widowati, 2017; Pujimulyani *et al.*, 2018; Widowati, Wargasetia, *et al.*, 2018).

Figure 2 The Hydrolysis Reaction of p-NPG

The anti-diabetic activity of ethanol extracts from lime peels due to the presence of phenolic compounds like flavonoids and tannins that can inhibit the carbohydrate-hydrolyzing enzymes. This statement was supported by the result of Bouabid et al. (2018). They reported that aqueous and methanol extract of *Atractylisgummifera* L. that was rich in the phenolic compound had anti-diabetic activity through inhibition of α-amylase, α-glucosidase, and β-galactosidase (Bouabid *et al.*, 2018).

The preceding study about the anti-diabetic activity of lime peel still not available yet. However, there are several studies that explore other pharmacologic activity of lime, and these were an antioxidant activity, repellant, and anti-microbial activity from the peel, seed, and fruit pulp of lime (Kusumaningrum, 2015; Apriliani, Ramadhan and Rijai, 2017; Stevenie *et al.*, 2019).

As a comparison, the following table would show the anti-diabetic activity of some types of orange peel, which was reported by Lim and Loh (2016) (Lim and Loh, 2016).

Table 5 The Anti-Diabetic Activity of Some types of orange

Sample	Solvent	Percent of Inhibition	
		α-Glucosidase Enzyme	α-amylase Enzyme
Citrus maxima	80% Acetone	38.17 ± 9.71	41.06 ± 10.94
	Ethyl Acetate	38.04 ± 2.01	30.26 ± 11.82
Citrus hystrix	80% Acetone	47.16 ± 11.32	25.47 ± 6.86
	Ethyl Acetate	43.80 ± 8.94	26.98 ± 6.54
C*itrus aurantifolia*	80% Acetone	53.95 ± 14.34	15.63 ± 3.93
	Ethyl Acetate	41.37 ± 5.45	39.97 ± 8.60
Citrus microcarpa	80% Acetone	61.79 ± 4.13	32.66 ± 9.17
	Ethyl Acetate	45.30 ± 5.35	43.99 ±22.03

The IC50 value of Lime peels (*Citrus amblycarpa*) was 125.93 ± 9.14 that was higher than other types of citrus peels that were shown in the table above. It means that the ability of *Citrus amblycarpa* as α-Glucosidase Enzyme inhibition was not as good as the other type of *Citrus*.

CONCLUSION

Hence the lime peel has the potential of anti-diabetic activity that has the highest anti-diabetic activity in the 50 µg/mL, and IC50 value ranged 125.93 ± 9.14 µg/mL, it is caused by the presence of some phytochemicals like flavonoid, phenol, steroid/triterpenoid, and alkaloid.

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