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ALPHA - AMYLASE INHIBITORY ACTIVITIES OF LIME (*Citrus amblycarpa* (Hassk.) Ochse) ETHANOL EXTRACT

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Abstract

This research was conducted to determine the activity test of the extract of lime peel (Citrus amblycarpa) with a concentration of 6, 25 µg / ml, 12, 5 µg / ml, 25 µg / ml, 50µg / ml, 100µg / ml, 200µg / ml to the α-amylase inhibitor. The purpose of this study was to observe the antidiabetic properties of lime peel extract (Citrus amblycarpa) by observing the inhibitory activity of α-amylase. This research was conducted with an experimental method with a post-test only design and sampling with a purposive sampling method. The activity test of lime extract (Citrus amblycarpa) against α-amylase inhibitors was carried out using a spectrophotometer observing the color changes that occur by measuring its absorbance. The results obtained in this study were carried out with the Post Hoc test. Then the analysis was continued with linear regression analysis to assess the IC_{50} for the α-amylase enzyme in the ethanol extract of lime peel (Citrus amblycarpa). The results obtained in the form of lime peel extract (Citrus amblycarpa) can inhibit α-amylase at the highest concentration of 200 µg / ml with inhibition percentage of 52, 88%. The IC_{50} value of the extract of lime (Citrus amblycarpa) against the α-amylase enzyme was 168.24 ± 21.04 µg / mL.

Keywords: ***lime peel extract (Citrus amblycarpa), α-amylase enzyme, α-amylase inhibitor***

INTRODUCTION

One of annual metabolic diseases with hyperglycemia as clinical condition is diabetes mellitus, which is a result of insulin secretion dysfunction and insulin dysfunction or both (Soelistijo *et al.*, 2015). Diabetes mellitus can increase blood sugar level, including those caused by carbohydrate consumption (Sabarina, 2016). Carbohydrate is one of the substance responsible for energy production by breaking it down to monosaccharides by α-amylase enzyme (Rahimzadeh *et al.*, 2014).

One of the ways in overcoming diabetes mellitus is by inhibiting the work of carbohydrate hydrolysis enzyme, including α-amylase enzyme (Manaharan, Palanisamy and Ming, 2012). Acarbose and Miglitol are some of the medication used in the medical field with various side effects, including flatulence, hypoglycemia, diarrhea and resistance to prolonged-use of drugs (Chopade *et al.*, 2012). Several natural ingredients can be alternatives, which works as α-amylase inhibitor, including red bean water extract (Wardani, 2018), rosella extract (Gondokesumo, Kusuma and Widowati, 2017), *Phaseolus vulgaris* extract (Barrett and Udani, 2011), methanol extract of *Cinnamomum zeylanicum, Artocarpus altilitis, Piper betel,* and *Artocarpus heterophyllus* (Nair, Kavrekar and Mishra, 2013), methanolic extract of *Sargassum hystrix* and *Eucheuma denticulatum* (Husni *et al.*, 2018).

Lime is a type of citrus which contains flavonoid, polyphenol, tannin, glycoside and essential oil (Putra *et al.*, 2018). Lime peel contains flavonoid, which is beneficial in decreasing blood sugar level (Li *et al.*, 2018). The essential oil content in citrus fruits revealed significant decrease in fasting blood glucose and liver glucose level (Uddin *et al.*, 2014). Studies on the activity of α-amylase inhibitor in lime ethanol extract should be conducted for diabetes mellitus treatment.

MATERIALS AND METHODS

Materials

The α-amylase enzyme from *Bacillus amylodiquiefaciens* (Sigma, A7595), starch solution (Sigma, 34117), iodine (Merck, B0664261 108), NaCl (Merck, K26354104), NaH_2PO_4 (OmniPur, 8210), Na_2HPO_4 (Sigma Aldrich, S7907), distilled water, pH meter (OHAUS Starter300 Portable), Spatula, Multiskan Go Reader (Thermo Fisher Scientific 1510), micro pipette (1-10 µL, 50-200 µL, 100-1000 µL) (Eppendorf), Tips (1-10 µL, 50-200 µL, 100-1000 µL) (NEPTUNE), 96 well-plates (TPP 92096), 15 mL falcon tube (SPL 50015), 50 mL falcon tube (SPL 50050), analytical balance (AXIS), 1,5 mL Eppendorf tube (SPL 60015-1), vortex (WiseMix VM-10).

The Making of Lime Extracts

Extraction was performed using maceration technique. Cleaned lime peels were dried then ground and immersed in 70% ethanol solvent every 24 hours. The filtrate was collected until clear, then 70% ethanol filtrate was evaporated to create 70% ethanol extract paste.

Assessment of α-Amylase Inhibitor Activity

First, 20 µL of starch was inserted in each well, then 60 µL buffer was added to each well. Sample of 10 µL was added to the sample and blank wells, then the plate was incubated for 3 minutes at 37^{o}C. Afterwards, 20 µL α-amylase enzyme was added to each well, then incubated again at 37^{o}C for 15 minutes. Enzymatic reaction was halted by the addition of 40 µL HCl to each well. Lastly, 10 µL lugol was added to each well. The absorbance of any color changes was measured using spectrophotometer with 630 nm wavelength.

The percentage of inhibitory activity was calculated using the following formula:

$$\% \text{ inhibition} = (S-C) \times 100 / (B-C)$$

C : absorbance without starch

S : absorbance of tested sample

B : absorbance without sample

Statistical Analysis

Data from this study were processed by One-Way ANOVA, followed by Post-hoc Tukey HSD with 95% confidence interval (α = 0.05). The results of α-amylase enzyme inhibitor activity assessment were presented in Mean ± SD %, then linear regression was conducted to analyzed the value of inhibition concentration 50 (IC_{50}).

RESULTS AND DISCUSSION

Activity of α-Amylase Inhibitor

The α-amylase enzyme is one of the enzymes produced in pancreas with the function to break down starch to produce α-limit dextrin which consists of the combination of maltose, maltotriose, and oligosaccharide branch (6-8 glucose units) consists of α-1-4 and α-1-7 bond and oligosaccharides with various length and α-configuration (de Sales *et al.*, 2012).

The inhibitory activity on α-amylase can be seen depending on concentration, whereas higher concentration has higher inhibitory activity. The highest α-amylase inhibitory activity was in 200 µg/mL concentration with inhibitory percentage of 52.88%. According to statistical analysis, the higher the concentration of lime extract, the higher the inhibitory activity in inhibiting α-amylase.

This was seen from the results of Post-hoc Tukey HSD marked by superscripts on the table below. Afterwards, linear regression follows to assess IC_{50} on α-amylase enzyme on lime peel ethanol extract (*Citrus am-*

blycarpa).

Table 1. Inhibitory activity of α-amylase (%) by lime peel extract

Final Concentration (µg/mL)	Inhibitory activity of α-amylase (%)
6.25	36.29 ± 0.39^{a}
12.5	37.32 ± 0.27^{a}
25	38.26 ± 0.27^{ab}
50	40.55 ± 1.00^{b}
100	44.21 ± 1.36^{c}
200	52.88 ± 2.10^{d}

*Data were presented in Mean ± SD. Different superscripts (a, b, ab, c, d) indicated significant difference ($p < 0.05$) of Tukey HSD Post-hoc test.

The table above revealed significant difference of inhibitory activity on α-amylase enzyme, whereas the higher the concentration of lime extract, the higher the inhibitory activity on α-amylase. The average inhibitory activity in inhibiting α-amylase at 200 µg/ml concentration was 52.88%.

Lime peel extract with 100 µg concentration revealed mean inhibitory activity in inhibiting α-amylase of 44.21%. In 50 µg concentration, the inhibitory activity was 40.55%. In 25 µ/ml, the mean inhibitory activity was 38.26%. In 12.5 µg/ml, mean inhibitory activity was 37.32%, and in 6.25 µg/ml, the inhibitory activity was 36.29%.

Table 2. IC_{50} value in α-amylase enzyme inhibition by lime peel extract

Sample	Equation	R^2	IC_{50} (µg/mL)	IC_{50} (µg/mL)
EKL (1st repetition)	y = 0.0881x + 35.893	0.99	160.12	168.24 ± 21.04
EKL (2nd repetition)	y = 0.0927x + 35.866	0.99	152.47	
EKL (3rd repetition)	y = 0.0702x + 36.512	0.99	192.14	
EKL (mean)	y = 0.0837x + 36.091	0.99	166.18	

The table above showed that the IC_{50} of lime peel extract (*Citrus amblycarpa*) on α-amylase enzyme was 168.24 ± 21.04 µg/mL. This showed that 189.28 – 147.20 µg/mL of lime peel ethanol extract concentration was needed to inhibit 50% of available α-amylase enzyme.

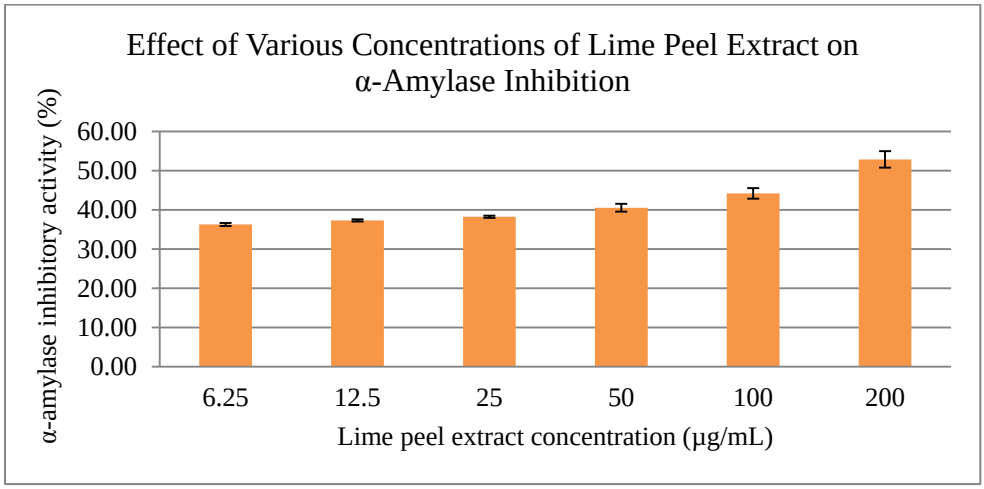

Figure 1. Effect of different concentrations of lime peel extract on α-amylase enzyme inhibition.

Alpha amylase (α-1,4-glucan-4-glucanohydrolase) is an enzyme that acts in randomly hydrolyze starch to maltose (P *et al.*, 2011). The inhibition of α-amylase has an important role in controlling diabetes because it can decrease glucose absorption (Adnyana *et al.*, 2016).

The method used was starch-iodine. Iodine as color indicator (Etoundi *et al.*, 2010). Non-hydrolyzed starch can form blue colored complex substance with iodine. The resulted blue color can be used as an indicator of sample ability as reaction inhibitor (Howard, 2015).

Lime peel extract (*Citrus amblycarpa*) has the potential of inhibitory activity through α-amylase assessment and showed high activity within 50-500 µg/ml concentration (Kim, Kwon and Son, 2000).

CONCLUSION

Lime peel extract (*Citrus amblycarpa*) has α-amylase inhibitory activity, seen from IC_{50} of 168.25 µg/ml and strong activity of 52.88% at 200 µg/ml concentration.

REFERENCES

1. Adnyana, I. K. *et al.* (2016) ‘ Pancreatic lipase and α-amylase inhibitory potential of mangosteen (Garcinia Mangostana Linn.) pericarp extract ’, *International Journal of Medical Research & Health Sciences*. doi: 10.5958/2319-5886.2016.00006.0.
2. Barrett, M. L. and Udani, J. K. (2011) ‘A proprietary alpha-amylase inhibitor from white bean (Phaseolus vulgaris): A review of clinical studies on weight loss and glycemic control’, *Nutrition Journal*. doi: 10.1186/1475-2891-10-24.
3. Chopade, B. A. *et al.* (2012) ‘Antidiabetic activity of gnidia glauca and dioscorea bulbifera: Potent amylase and glucosidase inhibitors’, *Evidence-based Complementary and Alternative Medicine*. doi:

10.1155/2012/929051.

4. Etoundi, C. B. *et al.* (2010) 'Anti-amylase, anti-lipase and antioxidant effects of aqueous extracts.', *Journal of Natural Products*.
5. Gondokesumo, M. E., Kusuma, H. S. W. and Widowati, W. (2017) 'α-/β-Glucosidase and α-Amylase Inhibitory Activities of Roselle (Hibiscus sabdariffa L.) Ethanol Extract', *Molecular and Cellular Biomedical Sciences*. doi: 10.21705/mcbs.v1i1.3.
6. Howard, W. (2015) 'Pengujian Aktivitas Enzim Alfa-Amilase', *Research Gate*.
7. Husni, A. *et al.* (2018) 'In vitro antidiabetic activity of sargassum hystrix and eucheuma denticulatum from yogyakarta beach of indonesia', in *Proceedings of the Pakistan Academy of Sciences: Part B*.
8. Kim, J. S., Kwon, C. S. and Son, K. H. (2000) 'Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid', *Bioscience, Biotechnology and Biochemistry*. doi: 10.1271/bbb.64.2458.
9. Li, K. *et al.* (2018) 'Inhibitory effects against α-glucosidase and α-amylase of the flavonoids-rich extract from Scutellaria baicalensis shoots and interpretation of structure–activity relationship of its eight flavonoids by a refined assign-score method', *Chemistry Central Journal*. doi: 10.1186/s13065-018-0445-y.
10. Manaharan, T., Palanisamy, U. D. and Ming, C. H. (2012) 'Tropical plant extracts as potential antihyperglycemic agents', *Molecules*. doi: 10.3390/molecules17055915.
11. Nair, S. S., Kavrekar, V. and Mishra, A. (2013) 'In vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts', *European Journal of Experimental Biology*.
12. P, S. *et al.* (2011) 'Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants', *BMC Complementary and Alternative Medicine*. doi: 10.1186/1472-6882-11-5.
13. Putra, G. M. D. *et al.* (2018) 'STANDARISASI DAN SKRINING FITOKIMIA EKSTRAK ETANOL 70% DAUN JERUK LIMAU (Citrus amblycarpa (Hassk.) Osche)', *Jurnal Kimia*. doi: 10.24843/jchem.2018.v12.i02.p15.
14. Rahimzadeh, M. *et al.* (2014) 'Evaluation of alpha- amylase inhibition by Urtica dioica and Juglans regia extracts', *Iranian Journal of Basic Medical Sciences*.
15. Sabarina, D. (2016) 'Aktivitas Penghambatan Enzim A-Glukosidase Dan A-Amilase Dari Ekstrak Daun Salam, Daun Pandan, Daun Jeruk Purut Dan Kombinasinya'.
16. de Sales, P. M. *et al.* (2012) 'α-amylase inhibitors: A review of raw material and isolated compounds from plant source', *Journal of Pharmacy and Pharmaceutical Sciences*. doi: 10.18433/j35s3k.
17. Soelistijo, S. A. *et al.* (2015) *Konsensus Pengendalian dan Pencegahan Diabetes Melitus Tipe 2 di Indonesia 2015, Perkeni*. doi: 10.1017/CBO9781107415324.004.

18. Uddin, N. *et al.* (2014) 'In vitro α-amylase inhibitory activity and in vivo hypoglycemic effect of methanol extract of Citrus macroptera Montr. fruit', *Asian Pacific Journal of Tropical Biomedicine*. doi: 10.12980/APJTB.4.2014C1173.
19. Wardani, N. A. K. (2018) 'Enzim α-Amilase Inhibitor Pada Ekstrak Air Kacang Merah (Phaseolus vulgaris L.) Untuk Penanggulangan Diabetes Melitus', *Jurnal Ilmu Pangan dan Hasil Pertanian*. doi: 10.26877/jiphp.v1i2.1900.