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The Inhibitory Effect of *α*-amylase Enzyme of Fraction and Isolate of Mareme Leaves (*Glochidion arborescens* Blume)

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Abstract

Mareme leaves (Glochidion arborescens Blume) is a plant that has potential as an antidiabetic agent. A previous study reported that ethanol extract of Mareme leaves exhibits active inhibitory activities of α-amylase enzyme. This study aimed to determine the α-amylase enzyme inhibitory activities of fraction and isolate from Mareme leaves. The ethanol extract of Mareme leaves was fractionated by using vacuum liquid chromatography. Then, it was isolated and purified using a chromatotron, a radial chromatographic method. The fraction and isolate were assayed for α-amylase enzyme inhibitory activities. The result indicated that Mareme leaves could inhibit an active α-amylase enzyme inhibitory activity with an IC_{50} value of 28.262 ppm. Mareme leaves isolate has a very active inhibition (IC_{50} value = 21.024 ppm) comparable with acarbose, a positive control (IC_{50} value = 19.486 ppm). In addition, the statistical analysis using the t-test also resulted in a significant difference in average inhibition of α-amylase enzyme between fraction and isolate of Mareme leaves. The study concludes that the fraction and isolate of Mareme leaves are potential inhibitors of α-amylase enzyme in reducing calorie intake. As such, this plant extract has been proven that it could be a potential antidiabetic or anti-obesity agent to prevent or treat chronic diseases.

Keywords

Glochidion arborescens; Antidiabetic; α-Amylase enzyme; Isolate

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1 Introduction

Diabetes mellitus (DM) is a metabolic disorder of the body typically characterised by high levels of blood sugar caused by damage or impaired insulin secretion[1]. Blood sugar levels that are too high will aggravate complications like nephropathy, neuropathy, and retinopathy. Using *α*-glucosidase inhibitors, such as acarbose, is one of the treatment methods for lowering blood sugar levels by inhibiting the *α*-amylase enzyme[2]. However, there are still issues such as high costs, drug safety, and side effects, including abdominal pain, flatulence, and diarrhea[3].

Mareme leaves contain antidiabetic properties. Based on previous studies, the ethanolic extract of Mareme leaves can reduce blood glucose levels by 72.2% in male mice for seven days after being given. The *α*-amylase enzyme inhibition assay of the ethanolic extract of Mareme leaves resulted in an IC_{50} value of 48.27 ppm. It was reported to have

stronger antidiabetic activity than acarbose, which has an IC_{50} value of 63.31 ppm[4,5].

It is known that the ethanol extract of Mareme leaves (*Glochidion arborescens* Blume) contains flavonoids and polyphenols that possess antioxidant activity[6]. According to previous research, the flavonoid compounds in the active fraction of *Peltophorum pterocarpum*'s leaves and bark, specifically quercetin-3-O-*β*-D-galactopyranoside, can inhibit the *α*-amylase enzyme and have a more significant inhibitory effect than acarbose[7]. Flavonoid compounds can inhibit the *α*-amylase enzyme by reducing fluctuations in plasma glucose and delaying the breakdown of disaccharides and polysaccharides into glucose in the intestine[8]. Flavonoids have antioxidant properties that can reduce sugar levels in the blood. Antioxidants protect pancreatic cells from free radicals generated in hyperglycemic conditions and can bind free radicals to reduce insulin resistance[9]. Antioxidant polyphenols such as curcumin and resveratrol can reduce pancreatic cell resistance and increase insulin secretion[10].

This study aims to determine the inhibition potential of the *α*-amylase enzyme of fraction and isolate from Mareme (*Glochidion arborescens* Blume) leaves.

2 Method

2.1 Preparation of Plant Material

Wet Mareme leaves were cleaned by washing with running water and then cut into small pieces. The leaves were then dried by using an oven at temperature that ranges between 30 to 50°C. The dried leaves were grinded into fine powder[11].

2.2 Extraction

800 g of powdered Mareme leaves were extracted using 95% ethanol. Subsequently, the solvent was replaced every 24 hours, and the extraction was repeated three times at 24 hours intervals. The liquid extract was concentrated by using a rotary evaporator and ~~a~~ water bath at 60°C[11].

2.3 Fractionation Using Vacuum Liquid Chromatography (VLC)

A total of 20 g of Mareme leaves extract (*Glochidion arborescens* Blume) was impregnated with 40 g of silica gel (0.063-0.200 mm). The stationary phase is silica gel 60 GF_{254}, and the mobile phase is n-hexane: ethyl acetate. With increasing solvent polarity, fractionation was performed by using various ratios of n-hexane eluent: ethyl acetate, including 9:1 (2x), 8:2 (3x), 7:3 (3x), 6:4 (2x), 5:5 (2x), and ethanol (2x). 175 mL of eluent was required for each elution. According to the ratio of eluents, different glass bottles were used to store each fraction. The fractions obtained were analyzed by TLC using a 7:3 ratio (n-hexane:ethyl acetate). After combining the collections of elution with the same R_f, these collections can be categorized as non-polar fraction, semi-polar fraction, and polar fraction. Each fraction was concentrated by removing the solvent using a vacuum rotary evaporator[12].

2.4 Isolation and Purification Using Radial Chromatography (Chromatotron)

Chromatotron plates were prepared with a thickness of 2 mm, by using a mixture of silica gel 60 PF_{254} containing 55 g of gypsum silica gel (Merck 7749), 120 mL of cold distilled water to form a slurry and dried overnight (24 hours) or until it is fully dried. The semi-polar fraction of 250 mg of Mareme leaves was dissolved in acetone and dripped onto the chromatotron plate with a dropper. The plate was eluted with a 9.5:0.5 ratio (n-hexane: ethyl acetate). The isolate was collected by using a vial and their TLC profiles were examined under a shortwave UV lamp at 254 nm[13].

2.5 α-Amylase Enzyme Inhibition Assay

This study used *α*-amylase enzyme from *Aspergillus oryzae* (Sigma), with potato starch as a substrate, an iodine indicator, and acarbose as the standard reference. Acarbose (positive control), fraction, and isolate of Mareme leaves (*Glochidion arborescens* Blume) with

different concentrations of 10, 15, 20, 25, and 30 ppm were used to assay the *α*-amylase enzyme inhibitory effects. The procedures are outlined in Figure 1. The equation $y = bx + a$ was established based on linear regression. It is used to determine the IC_{50} values of tested samples. The percentages of inhibition obtained are summarized as shown in Table 1 by using Equation 1 below[14]:

$$\% \text{ Inhibition} = \frac{As - Ac}{As} x\ 100\% \qquad (1)$$

where *Ac* refers to absorbance of the control and *As* refers to absorbance of the sample.

2.6 Statistical Analysis

Using the SPSS program, the results of *α*-amylase inhibitory activities of fraction and isolate were analysed using the average *t*-test or *t*-test with a 95% confidence level. Figure 2 shows the overall research flow chart of this study.

3 Result and Discussion

Mareme leaves fine powder, a green-coloured powder has a tea-like aroma and a bitter taste. Using the maceration technique, 800 g fine powdered Mareme leaves (*Glochidion arborescens* Blume) were extracted using 95% ethanol solvent and yielded 11.94% (95.52 g) concentrated extract. Maceration is one of the simple, inexpensive, and thermolabile compound's safe extraction methods[15]. This extract was further fractionated using VLC and obtained 14 fractions and 3 sub-fractions, which are categorised as non-polar fraction (3.75 g), semi-polar fraction (3.90 g), and polar fraction (3.25 g).

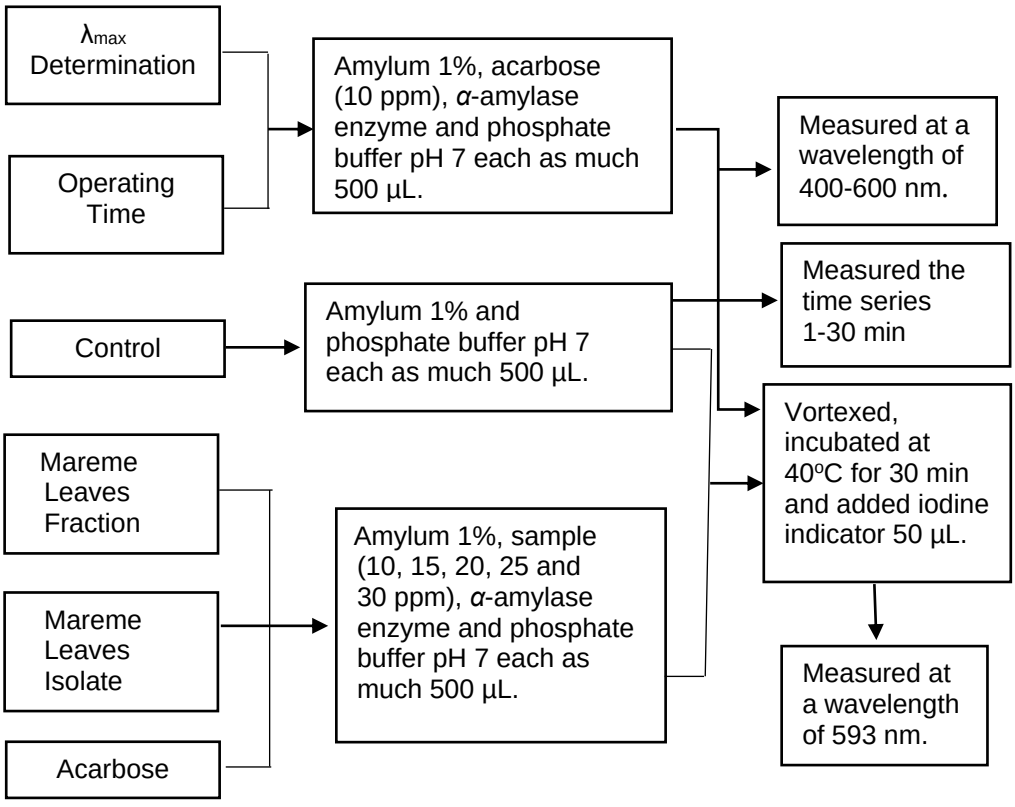


Figure 1. Flow chart of experiment procedures of *α*-amylase enzyme inhibition assay.

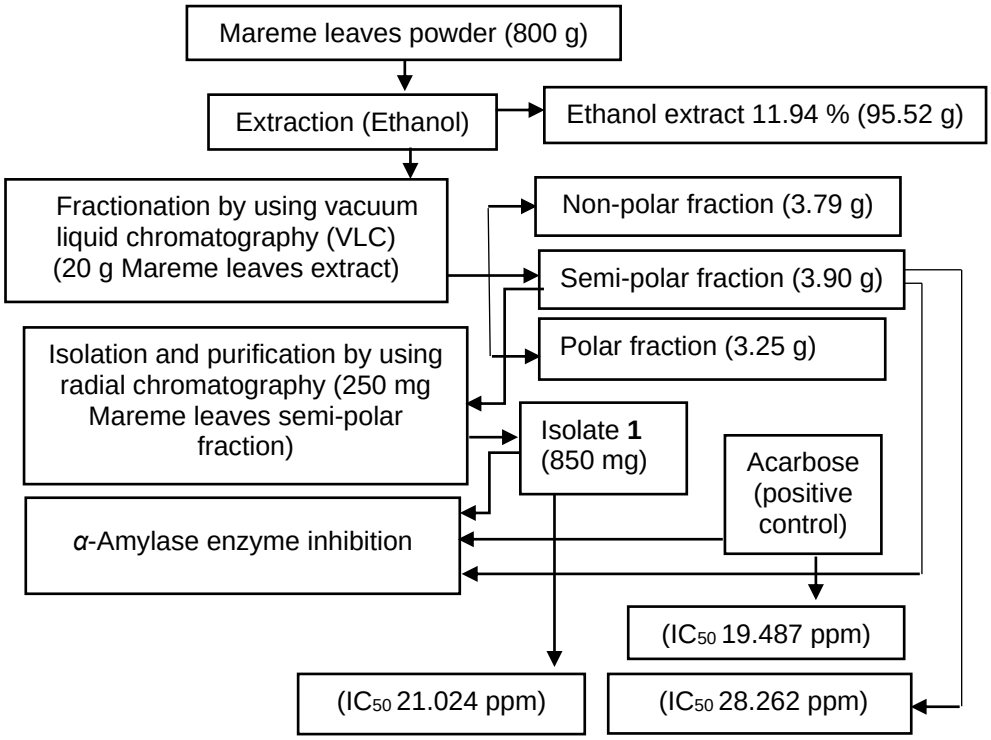


Figure 2. Flow chart of research process.

A chromatotron was used to isolate the semi-polar fraction of Mareme leaves. Semi-polar solvents attract polar and non-polar compounds, suggesting that the semi-polar fraction contains more secondary metabolites[9]. Based on the chromatographic separation method using a chromatotron, 850 mg of Mareme leaves isolate **1** was obtained.

In this study, *α*-amylase was used. The enzyme *α*-amylase catalyses the hydrolysis of starch into oligosaccharides, maltose, and glucose. The *α*-amylase enzyme belongs to the exo-hydrolase group of enzymes, such as hydrolysis of 1,4-glycosidic bonds to produce maltose[16]. Inhibition of the *α*-amylase enzyme can slow the digestion of carbohydrates, thereby decreasing the blood glucose absorption rate[17]. The *α*-amylase inhibition assay was carried out to determine the ability of the *α*-amylase enzyme to hydrolyse starch into simple sugars[18].

The inhibition of the *α*-amylase enzyme was measured by using a UV spectrophotometer at λ_{max} 593 nm. The results for max differ from those of previous studies in which λ_{max} is obtained at 565 nm. Differences in the λ_{max} results can be attributed to the type of spectroscopy and the purity of reagents[14]. The operating time of the remaining starch was determined for 0-30 min, and the absorbance was measured every 1 min. Operating time is the measurement time required to obtain a stable absorbance so that the absorbance does not fluctuate or remain constant over a specified period. Operating Time measurements reveal that the absorbance remains consistent from the second to the ninth minute.

To maintain the stability of the *α*-amylase enzyme, a pH 7 phosphate buffer was added into the tested sample. The pH plays a significant role in the stability of the *α*-amylase enzyme. The optimal pH range for *α*-amylase activity was between 6.5-7.5. After the *α*-amylase enzyme ended the starch substrate reaction, the absorbance can be measured[19]. The absorbance value was obtained from the reaction of starch and iodine, which formed a blue complex with a blue-purple colour complex because the *α*-amylase enzyme did not hydrolyse it due to the presence of compounds in the sample that

inhibited its hydrolysis[20]. Amylose is characterised by a single helix, similar to cyclodextrin, which has hydrophobic properties, allowing it to bind to two molecules of iodine atoms. The blue colour is caused by the donor-acceptor interaction between water and electron-poor polyiodides[21].

The level of *α*-amylase inhibitory power is classified into four categories: very active if the $IC_{50} \leq 25$ ppm, active if 25 ppm < $IC_{50} \leq 50$ ppm, less active if 50 ppm < $IC_{50} \leq 100$ ppm, and inactive if the $IC_{50} > 100$ ppm[22]. The % inhibition of the enzyme *α*-amylase Mareme leaves fraction, isolate, and acarbose are tabulated in Table 1, while that of IC_{50} values are shown in Table 2.

Table 1. Percentage inhibition of *α*-amylase enzyme of Mareme leaves semi-polar fraction, isolate and acarbose.

Concentration (ppm)	Mean ± SD (ppm)	% Inhibition	Equation
Mareme leaves semi-polar fraction			
10	0.264 ± 0.015	19.939	$y = 1.5792x + 5.3678$ $R^2 = 0.9856$
15	0.308 ± 0.019	30.519	
20	0.355 ± 0.030	36.309	
25	0.401 ± 0.013	46.633	
30	0.440 ± 0.003	51.363	
Control	0.214 ± 0.008	19.939	
Mareme leaves isolate **1**			
10	0.356 ± 0.013	37.640	$y = 0.9968x + 29.0428$ $R^2 = 0.9754$
15	0.403 ± 0.008	44.913	
20	0.443 ± 0.010	49.887	
25	0.492 ± 0.005	54.878	
30	0.527 ± 0.006	57.578	
Control	0.222 ± 0.010		
Acarbose			
10	0.345 ± 0.028	38.260	$y = 1.0203x + 30.117$ $R^2 = 0.9379$
15	0.395 ± 0.011	46.075	
20	0.457 ±0.022	53.391	
25	0.486 ± 0.023	56.172	
30	0.516 ± 0.012	58.720	
Control	0.213 ± 0.006		

Note: SD refers to standard deviation

Table 2. IC_{50} of *α*-amylase enzyme of Mareme leaves semi-polar fraction, isolate and acarbose.

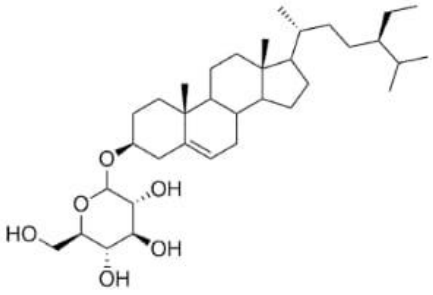
Samples	IC_{50} (ppm)	*α*-amylase inhibitory activity
Mareme leaves semi-polar fraction	28.262	Active
Mareme leaves isolate **1**	21.024	Very active
Acarbose (positive control)	19.487	Very active

Note: $IC_{50} \leq 25$ ppm: Very active; 25 ppm < $IC_{50} \leq 50$ ppm: Active; 50 ppm < $IC_{50} \leq 100$ ppm: Less active; $IC_{50} > 100$ ppm: Inactive

Based on % inhibition of the enzyme *α*-amylase, the isolate of Mareme leaves (Table 1) had an inhibition equivalent to the comparison of inhibition of acarbose (Table 1) with a very active inhibitory power due to the $IC_{50} \leq 25$ ppm. Acarbose was used as a comparison because it is a drug used to control blood sugar levels and

can inhibit the enzyme *α*-amylase's activity. Acarbose functions by inhibiting the digestive system's hydrolysis of disaccharides and carbohydrates. Acarbose can also inhibit the transformation of sucrose into glucose and fructose[18,23]. The findings also indicated that the Mareme leaves fraction possessed an active inhibitory power, as its IC_{50} ranged between 25 and 50 ppm (Table 2). An equation of linear regression if the R^2 value is close to 1. The isolate of Mareme leaves has a correlation coefficient close to 1, with an R^2 value of 0.9856. The results showed that the % inhibitions strongly correlate to Mareme leaves fraction.

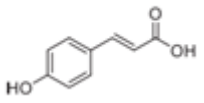
A previous study stated that Mareme leaves extract inhibited the *α*-amylase enzyme at a concentration of 40 ppm with an IC_{50} value of 48.27 ppm[5]. Another study conducted on an isolate of *Syzygium cumini* leaves against the inhibition of the *α*-amylase enzyme resulted to an IC_{50} value of 39.9 ppm, classifying it as active inhibition[24]. According to Dehghan et al.[25], *Rheum turkestanicum* isolate, namely daucosterol (**2**), could inhibit the *α*-amylase enzyme with an IC_{50} value of 46.4 ppm and an active inhibition category.



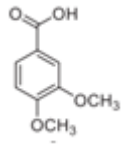
(**2**)

Figure 3. Structure of an isolate compound from roots of *Rheum turkestanicum*.

p-Coumaric acid (**1**), 3,4-dimethoxy benzoic acid (**3**), and 4-heptyloxy benzoic acid (**4**) isolated from *Ziziphus oxyphylla* fruit each had *α*-amylase enzyme inhibitory activity with an IC_{50} of 85 ppm, 112 ppm, and 110 ppm[26].



1



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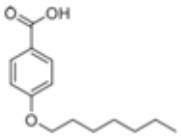

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Figure 4. Structures of isolated compounds (**1**) *p*-coumaric acid, (**3**) 3,4-dimethoxy benzoic acid, (**4**) 4-heptyloxy benzoic acid from roots of *Ziziphus oxyphylla*.

The semi-polar fraction and isolate (**1**) of Mareme leaves (*Glochidion arborescens* Blume) can inhibit the *α*-amylase enzyme. The IC_{50} values of Mareme leave fraction and isolate were lower compared to previous studies. The lower the IC_{50} value, the greater the *α*-amylase enzyme inhibition.

Based on the *t*-test analysis, the average inhibition value of the *α*-amylase enzyme in Mareme leaves fraction sample was 28.679. In contrast, the average inhibition value of *α*-amylase enzyme in the Mareme leaves isolate was 20.887. A sig (2-tailed) value of $p = 0.002$ (< 0.05). There was a significant difference in the *α*-amylase inhibitory activities between the semi-polar fraction and the isolate of Mareme leaves (*Glochidion arborescens* Blume).

4 Conclusion

Based on the findings in this study, it can be concluded that the fraction (semi-polar) and isolate **1**, namely *p*-coumaric acid of Mareme leaves (*Glochidion arborescens* Blume), can inhibit the *α*-amylase inhibitory activity. Mareme leaves isolate has a very active inhibitory power to inhibit the *α*-amylase enzyme with an IC_{50} value of 21.024 ppm and can

be considered equivalent to acarbose with an IC_{50} value of 19.487 ppm.

The study concludes that the fraction and isolate of Mareme leaves are potential inhibitors of the *α*-amylase enzyme in reducing calorie intake. As such, this plant extract could be a potential antidiabetic or anti-obesity agent to help in the prevention or treatment of chronic diseases.

Conflict of Interest

No conflict of interest.

Acknowledgment

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Author Contribution

Conceptualization: Ambarsari, N. & Haryoto
Data curation: Ambarsari, N.
Methodology: Ambarsari, N. & Haryoto
Formal analysis: Ambarsari, N.
Visualisation: Ambarsari, N.
Software: Ambarsari, N.
Writing (original draft): Ambarsari, N.
Writing (review and editing): Ambarsari, N. & Haryoto
Validation: Haryoto
Supervision: Haryoto
Funding acquisition: Ambarsari, N.
Project administration: Ambarsari, N.

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