



ORIGINAL ARTICLE

# From Nature to Laboratory: The Impact of Leilem Leaves' Ethanol Extract on Pancreatic Lipase Enzyme Activity

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## Abstract

For many years, there have been theories on the possibility of preventing or delaying type 2 Diabetes Mellitus by altering some of its risk factors. Drugs that combat obesity have recently been researched concerning the prevention of type 2 Diabetes Mellitus. Inhibition of the digestive enzyme pancreatic lipase is a potential therapeutic strategy in treating and managing chronic diseases such as diabetes and obesity. Plants containing bioactive compounds are identified as potential sources of pancreatic lipase enzyme inhibitors. The use of natural compounds in inhibiting pancreatic lipase enzyme activity is considered to have more potential due to low toxicity and side effects. This study aims to determine the potential and activity effect of ethanol extract of leilem leaves on inhibiting pancreatic lipase enzyme. This study is a laboratory experimental study, the method of measuring lipase inhibition potential was performed using porcine lipase and PNPB with several modifications and using Orlistat as a positive control. Readings were taken using an ELISA reader at a wavelength of 405 nm. The data were then processed to obtain the IC<sub>50</sub> value and relative potency. The results of in vitro studies have shown the potential of leilem leaf extract to inhibit pancreatic lipase enzyme activity. Qualitatively, the results showed that leilem leaf extract contains secondary metabolite compounds such as Alkaloids, Flavonoids, Tannins, Saponins, Steroids, and Terpenoids. Quantitatively, the results showed that the ethanol extract of leilem leaves had an absorbance value at the lowest concentration of  $1.346 \pm 0.53$  and  $0.709 \pm 0.29$  for the highest concentration. The IC<sub>50</sub> result obtained was 137.89 µg/mL while the IC<sub>50</sub> value of the positive control Orlistat was 77.022 µg/mL. Ethanol extract of leilem leaves (*Clerodendrum minahassae* Teijsm. & Binn) has a potential value of 0.558.

## Introduction

The development of modern society has had a major impact on medicine and healthcare. Over the past hundred years, the development and manufacture of chemically synthesized medicines have transformed healthcare worldwide. However, many healthcare services in developing countries still rely on traditional medicine and herbal medicines as the main way to cure patients. Herbal medicines are most commonly used in health promotion for disease prevention and therapy in chronic diseases such as cancer, cardiovascular disease, diabetes, and depression [1,2].

Health data released by the Global Burden of Disease in 2019 reported that there were 1.55 million deaths and 70 million disability rates due to diabetes mellitus [3]. Obesity is one of the risk factors for diabetes mellitus. Fat tissue affects metabolism by releasing hormones, glycerol, leptin, cytokines, adiponectin, and proinflammatory substances, as well as the release of nonesterified fatty acids (NEFA). In obese individuals, the levels of these substances increase [4]. A previous study showed that pancreatic amylase and lipase function are impaired in type 2 patients with diabetes, and this observation is particularly important in type 2 diabetes. It has

been suggested that the analysis of pancreatic enzymes in diabetic patients may be a useful parameter in determining the progression of the disease [5].

For many years, there have been theories on the possibility of preventing or delaying type 2 diabetes mellitus by altering some of its risk factors. Drugs that combat obesity have recently been researched concerning the prevention of type 2 diabetes mellitus. Inhibition of the digestive enzyme pancreatic lipase is a potential therapeutic strategy in treating and managing chronic diseases such as diabetes and obesity [6]. The process of inhibiting pancreatic lipase enzyme affects fat decomposition so that the fat entering the blood can be controlled [7]. Plants with bioactive compounds were identified as potential sources of pancreatic lipase enzyme inhibitors [8]. In a study conducted by Zhang et al., it was found that the flavonoid nucleus is a necessary structure in the inhibitory activity of pancreatic lipase enzymes [9].

One of the plants that have this content is *Clerodendrum minahassae* Teijsm. & Binn, known as leilem [10,11]. Leilem leaves contain chemicals such as alkaloids, saponins, flavonoids, steroids, phenols, and terpenoids that are efficacious as natural antioxidants, anti-inflammatory, antidiabetic, antimicrobial, reduce the risk of coronary heart disease and cancer, and can increase body immunity and stamina [12–14]. The use of natural compounds in inhibiting pancreatic lipase enzyme activity is considered to have more potential due to low toxicity and side effects.

However, the potential benefits of Leilem leaf extract in inhibiting pancreatic lipase enzymes are not well known. The abundant availability of leaves adds to the urgency of Leilem leaf extract research to maximize the utilization of herbal plants in the North Sulawesi region. In vitro studies were conducted to determine the initial potential and activity effect of ethanol extract of Leilem leaves on inhibiting pancreatic lipase enzyme. Sampling was conducted at the origin of leilem in Minahasa Regency to obtain quality plants in endemic areas.

## Materials and Methods

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### Materials

The materials used are leilem leaves, sulfuric acid ( $H_2SO_4$ ) 2N, Wagner reagent, Mayer reagent, Dragendorff reagent, ammonia, chloroform,  $Na_2HCO_3$ , hydrochloric acid (HCl) 1%, magnesium powder (Mg powder), concentrated sulfuric acid ( $H_2SO_4$ ),  $FeCl_3$  10% & 5%, and ethanol ( $C_2H_5OH$ ) 70% and 96%, 4-nitrophenyl butyrate, pancreatic lipase enzyme, buffer solution, orlistat (purity > 98%), DMSO, and distilled water.

### Sample Preparation

The samples taken in this study were the leaves of the leilem plant (*Clerodendrum minahassae* Teijsm. & Binn) which were sourced in two different locations, namely in Kayuuwi Village, West Kawangkoan Subdistrict, Minahasa Regency, North Sulawesi Province located at an altitude of 700 meters above sea level with coordinates  $1^{\circ}12'31''$  north latitude (LU) &  $124^{\circ}46'12''$  east longitude (BT) and in Rumoong Atas Village, Tareran Subdistrict, South Minahasa Regency, North Sulawesi Province located at an altitude of 600 meters above sea level with coordinates  $1.2210676^{\circ}$  north latitude (LU) and  $124.7367856^{\circ}$  east longitude (BT). The leaf samples used were young leaves near the shoots that were not perforated or damaged.

### Simplicia and Ethanol Extract of Leilem Leaves Preparation

Leilem leaves (*Clerodendrum minahassae* Teijsm. & Binn) that have been selected according to the criteria undergo a 14-day washing and drying process at room temperature. The process continues with dry sorting to remove and separate dirt, foreign objects, or damaged parts from the samples. After that, the dried leaf samples are blended into a fine powder with a fineness level of 100 mesh.

Leilem leaves were extracted using the maceration method. Leilem leaf powder was weighed as much as 400 grams and then poured with 2L of 96% ethanol solvent (1:5 ratio). The

maceration process was done with 96% ethanol solvent for 3×24 hours. Then, the remaceration process was carried out for 2×24 hours. The extraction process of maceration results was carried out by evaporation technique using an oven at 40 degrees for three days [15].

#### *Qualitative Phytochemical Compound Screening Ethanol Extract of Leilem Leaves*

Qualitative phytochemical screening was done using 2 g of condensed extract of leilem leaves homogenized with 20 mL of 70% ethanol. The resulting filtrate was tested with several standard methods commonly used to identify the presence of certain functional groups (secondary metabolite compounds).

**Alkaloids:** the extract was mixed with 5 mL of chloroform and 5 mL of ammonia and then heated. The filtered extract added five drops of 2 N sulfuric acid into each filtrate, then shaken and let stand. The solution will form two layers, separating the top of each filtrate to be tested with reagents in different test tubes. The tube I is dripped with Mayer reagent. The reaction will be called positive if a white precipitate is formed. The tube II is dripped Wagner's reagent. The reaction will be called positive if a brown precipitate is formed. In tube III, drop Dragendorff reagent. The reaction will be called positive if an orange precipitate is formed [15].

**Flavonoids:** the extract was mixed with 70% ethanol as much as 3 mL. After passing through the heating and filtering process, the filtrate is then added with magnesium powder (Mg) as much as 0.1 g and two drops of concentrated HCL. The test is positive if a red color forms in the ethanol layer indicating the presence of flavonoid compounds [15].

**Tannin:** the test was carried out by filtering the extract with 10 mL of distilled water. The filtrate formed is diluted with water until colorless. After that, 2 mL of the solution was mixed with two drops of FeCl<sub>3</sub> 1%. The reaction is positive if a greenish-brown or blackish-blue color is formed in the solution. The reaction indicates that the extract contains tannin compounds [15].

**Saponins:** the test was carried out by putting the extract into a test tube and adding 10 mL of hot water. The solution goes through a cooling and shaking process for 10 seconds. The reaction is positive if a froth is formed with a height of 1-10 cm for not less than 10 minutes, and when one drop of HCL 2 N is added, the froth formed does not disappear [15].

**Steroids and Triterpenoids:** the test was carried out by mixing the extract with 3 mL of 70% ethanol or 3 mL of chloroform, then adding 2 mL of concentrated sulfuric acid and 2 mL of anhydrous acetic acid. Positive reactions contain steroid compounds if there is a color change from purple to blue or green. If a brownish color forms between the surfaces, the extract is positive for terpenoid compounds [15].

#### *Pancreatic Lipase Enzyme Inhibitory Activity Test*

Pancreatic lipase hydrolyzes p-nitrophenyl, produces a yellow color in an aqueous solution, and has a maximum absorption peak at 405 nm. The lipase inhibitory activity of the extracted sample was evaluated by measuring the amount of PNP at 405 nm. The assay method was based on the previously published method by Kim et al. [16], plus some modifications. The assay required 20 µL of substrate (10 mM 4-nitrophenyl butyrate in ethanol), 40 µL of test samples with serial concentrations of 10 µg/mL, 20 µg/mL, 40 µg/mL, and 60 µg/mL dissolved in DMSO solution, positive control on the test sample using Orlistat with the same concentration series, then 40 µL of pancreatic lipase enzyme that has been dissolved in buffer solution (2.5 mg/mL enzyme mixed in 0.1M phosphate buffer pH 8.0). The mixture was then incubated for 20 minutes at 37°C. 50 µL acetone solution was used as a stop solution. Then, the absorbance value was measured at a wavelength of 405 nm using an ELISA reader. All analyses were performed in three repetitions, and the results were presented as percentage inhibition (% I), as shown in Equation 1 [17].

$$\% I = \frac{(\text{Absorbance of Blank} - \text{Absorbance of the Sample Test})}{\text{Absorbance of Blank}} \times 100\% \quad (1)$$

The ability of the extract to inhibit pancreatic lipase enzyme activity is expressed as  $IC_{50}$  (inhibition concentration 50%). The likelihood of a sample or extract inhibiting pancreatic lipase enzyme activity comparable to a positive control such as Orlistat is expressed as relative potency. The relative potency was calculated using the Equation 2 [17].

$$\text{Relative Potency} = \frac{IC_{50} \text{ Value Of Orlistat}}{IC_{50} \text{ Value of Leilem Leaf Ethanol Extract}} \quad (2)$$

## Results and Discussion

### *Leilem Leaf (Clerodendrum minahassae Teijsm. & Binn) Extraction Results*

Simplisia was produced by taking 5 kg of leilem leaf samples. After passing the wet sorting and drying process, 970.59 g of dry leaves, ready to be mashed, were obtained. The dried simplisia was then pulverized with a 100 mesh sieve to obtain 716.32 g of fine simplisia powder. The results of the extraction of 400 g of fine simplisia from leilem leaves by maceration using 2L of 96% ethanol solvent and re-maceration using 500 ml of 96% ethanol solvent were 28.84 g of thick extract of leilem leaves (*Clerodendrum minahassae* Teijsm. & Binn). The percentage value of the yield obtained is 7.21%.

### *Phytochemical Compound Screening of Ethanol Extract of Leilem Leaf*

The results of screening phytochemical compounds (secondary metabolites) on ethanol extracts of Leilem leaves (*Clerodendrum minahassae* Teijsm. & Binn) provide an overview of the content of secondary metabolite compounds in the extract and are shown in Table 1. In the screening results of phytochemical compounds, it was found that the ethanol extract of leilem leaves in this study contained alkaloid compounds, flavonoids, tannins, saponins, steroids, and terpenoids. This is to the previously discussed theory that the source of natural materials that can be utilized as natural inhibitors of pancreatic lipase enzymes are plants that have active substances such as polyphenols, flavonoids, saponins, terpenoids, and alkaloids [8]. In previous studies, polyphenolic compounds were found to positively impact fat metabolism pathways. These compounds can inhibit the activity of pancreatic lipase, lipoprotein lipase (LPL), and glycerophosphate dehydrogenase (GPDH) enzymes. Extracts containing polyphenols can reduce body weight, fat content, plasma-free fatty acid (FFA) levels, and fat accumulation in the liver. In a study conducted by Zhang et al., it was found that the flavonoid nucleus is a necessary structure for the inhibitory activity of the pancreatic lipase enzyme [8,9].

**Table 1.** Phytochemical Screening Result of Ethanol Extract of Leilem (*Clerodendrum minahassae* Teijsm. & Binn).

Phytochemicals	Result
Alkaloids	Positive (+) Positive (+) Positive (+)
Flavonoids	Positive (+)
Tannin	Positive (+)
Saponnis	Positive (+)
Steroids and Triterpenoids	Positive (+)

Description:

(+) Positive / Compound contained in the extract

(-) Negative / Compound not contained in the extract

### *Analysis of Pancreatic Lipase Enzyme Inhibitory Activity*

Determination of the inhibitory potential of natural compounds can be done by various enzyme assay methods, one of which is the light absorption-based detection method. The in vitro uptake

assay was evaluated by measuring the amount of p-nitrophenyl hydrolyzed at 405 nm uptake. The assay was performed using a modified standard method using a concentration series of ethanol extract of Leilem leaves and read at a wavelength of 405 nm. The test results were carried out in three repetitions (replication in 3 wells), and then the absorbance calculation results were presented in the average value  $\pm$  standard deviation. The average absorbance value of each concentration was used for further data processing. The data of absorbance values of serial concentrations of ethanol extract of leilem leaves are shown in detail in Table 2.

**Table 2.** Absorbance value data of leilem leaf extract (*Clerodendrum minahassae* Teijsm, & Binn) at 405 nm wavelength.

Concentration ( $\mu\text{g/ml}$ )	Mean $\pm$ SD
10	1.346 $\pm$ 0.53
20	1.321 $\pm$ 0.30
40	0.853 $\pm$ 0.27
60	0.709 $\pm$ 0.29

Orlistat was used as a positive control (comparator) in this study. Several previous studies have shown a significant effect of Orlistat formulation on lipase inhibitory activity. Orlistat (tetrahydrolipstatin) is also used as a drug for weight loss therapy due to its effect in preventing fat metabolism. The absorbance values of the Orlistat concentration series are detailed in Table 3.

**Table 3.** Absorbance value data of Orlistat at 405 nm wavelength.

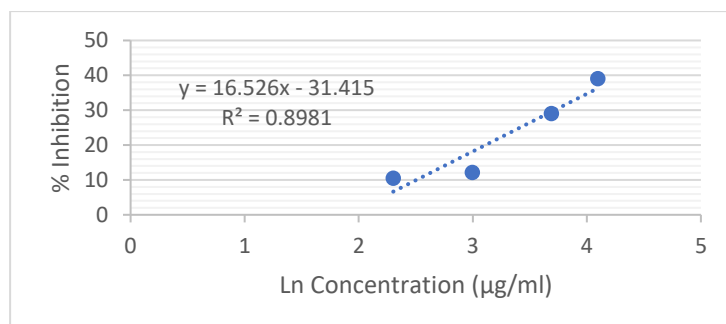
Concentration ( $\mu\text{g/ml}$ )	Mean $\pm$ SD
10	0.918 $\pm$ 0.26
20	0.877 $\pm$ 0.02
40	0.828 $\pm$ 0.10
60	0.754 $\pm$ 0.09

The absorbance values in both test solutions tend to decrease linearly when the concentration of the extract is increased. The absorbance increases initially due to the rapid color change of the dye, which reflects the high level of enzyme activity as they rapidly bind and convert the substrate into the final product. The absorbance decreases as the enzyme activity decreases due to the inhibition reaction. The inhibition mechanism generally explains that there is an interaction between the inhibitor and the substrate-enzyme binding. In other words, the inhibitor will bind to the free enzyme or substrate-enzyme complex near the substrate binding [18]. This indicates that pancreatic lipase enzyme performance is inhibited in the sample solution.

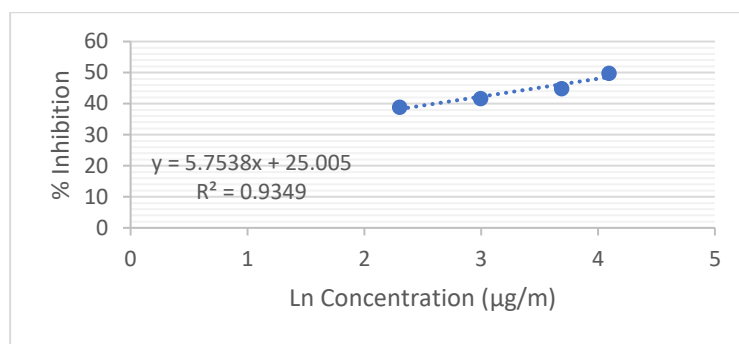
The calculation of the  $\text{IC}_{50}$  value was determined from the least squares regression equation of the logarithmic concentration (Ln Concentration) plotted against the percentage inhibition (%I). This value corresponds to the test sample concentration that can reduce the enzyme activity by 50% compared to the control. The percentage of lipase inhibition was calculated using Equation 1. After obtaining the results of lipase inhibition (%I) through the equation, then the results of the percentage of inhibition (%I) were plotted with logarithmic concentration to calculate a simple linear regression. The ability of the extract to inhibit pancreatic lipase enzyme activity is expressed as  $\text{IC}_{50}$ . The  $\text{IC}_{50}$  value is obtained from the exponential calculation of the x value after replacing  $y = 50$ . The likelihood of a sample or extract inhibiting pancreatic lipase enzyme activity comparable to a positive control such as Orlistat is expressed as relative potency.

From the calculations that have been carried out, a linear equation of the inhibitory activity of ethanol extract of Leilem leaves is produced as follows:  $y = 16,526x - 31.415$ . The standard curve graph for calculating the  $\text{IC}_{50}$  value of leilem leaf (*Clerodendrum minahassae* Teijsm. & Binn) ethanol extract is shown in Figure 1. From the calculation, the  $\text{IC}_{50}$  value of leilem leaf ethanol extract was 137.89  $\mu\text{g/ml}$ . Meanwhile, the linear equation value and the calculation of

the IC<sub>50</sub> value of Orlistat (positive control) are shown as follows:  $y = 5.7538x + 25.005$ . The standard curve graph for calculating the IC<sub>50</sub> value of Orlistat positive control is shown in Figure 2. From the calculation, the IC<sub>50</sub> value of Orlistat (positive control) was 77,022 µg/ml. The calculation results of pancreatic lipase enzyme inhibitory activity and IC<sub>50</sub> value are shown in Table 4.



**Figure 1.** Standard curve graph of leilem leaf (*Clerodendrum minahassae* Teijsm. & Binn) ethanol extract.



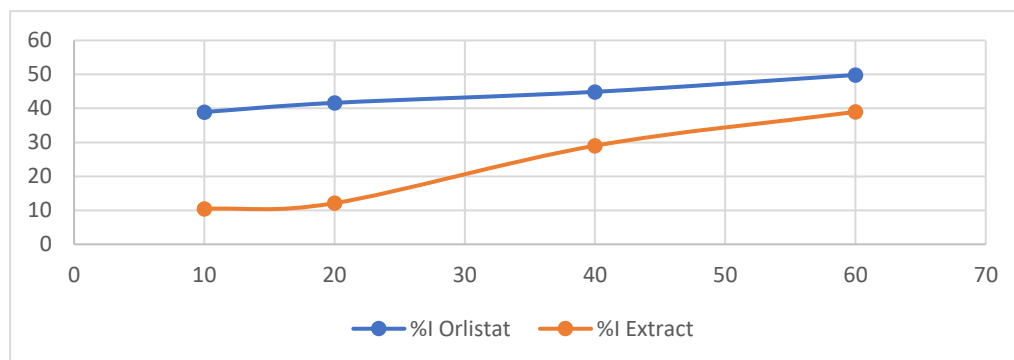
**Figure 2.** Standard curve graph of Orlistat.

The data in Table 4 shows a difference in the effect between the inhibitory activity of the Orlistat test sample and the ethanol extract of leilem leaves (*Clerodendrum minahassae* Teijsm. & Binn) at the same concentration. A comparison of the inhibition percentage curve of the Leilem leaf extract solution with the positive control Orlistat is shown in Figure 3. It can be seen that the visualization of the enzyme inhibitory activity of the positive control Orlistat is higher than that of the leilem leaf ethanol extract solution. This shows that in the same concentration, the potential inhibition of pancreatic lipase enzyme activity of ethanol extract is still below the drug Orlistat, so it is not possible to replace Orlistat as one of the treatment therapies for obesity and as a risk factor for diabetes.

The inhibitory activity of pancreatic lipase enzyme by Orlistat as a positive control is related to its role as an irreversible lipase inhibitor. Orlistat works by covalently binding to serine residues of gastric and pancreatic lipases. The pharmacological activity of Orlistat is dose-dependent. This can be explained by a simple E<sub>max</sub> model, which shows a steep initial part of the dose-response curve with a subsequent plateau for doses above 400 mg per day. This study had limited dose variation of the sample solution and positive control.

This is also supported by the basic theory that explains that in the process of pancreatic lipase enzyme reaction, amino acids serine (Ser152), aspartic acid (Asp176), and histidine (His263) play a role in maintaining the hydrolysis activity of pancreatic lipase. The amino acid serine (Ser152) is essential and responsible for lipolysis activity. The positive control used, Orlistat, is an irreversible lipase inhibitor that covalently binds to lipase residue Serine 152, which causes inhibition of gastric and pancreatic lipase *in vitro* and *in vivo* [16,19]. Meanwhile, this study did not test the mechanism and type of inhibitor for ethanol extract of leilem leaves. More specific

research is needed to test the mechanism and type of inhibitors that play a role in the leilem leaf extract.



**Figure 3.** Comparison chart of pancreatic lipase enzyme inhibitory activity (%) between Orlistat (positive control) and ethanol extract of leilem leaf (*Clerodendrum minahassae* Teijsm. & Binn).

Research by Buchholz and Melzig explained that flavonoids have great potential to inhibit pancreatic lipase enzymes. The inhibitory effect of active flavonoids depends on their structure, especially on the number and position of phenolic hydroxyl groups [20]. Flavones show more potent inhibitory activity than other types of flavonoids. The binding affinity of flavones determines their inhibitory activity against the pancreatic lipase enzyme [21]. In this study, the identification test of phytochemical compounds was only qualitative, and no quantitative test was carried out on flavonoid levels and further identification of the type of flavonoids contained in the ethanol extract of leilem leaves.

**Table 4.** Calculation results of Pancreatic Lipase Enzyme Inhibitory Activity and IC<sub>50</sub> Value.

Concentration (µg/mL)	Ln Concentration	Percentage Inhibition Orlistat	IC <sub>50</sub> Orlistat	Percentage Inhibition Extract	IC <sub>50</sub> Extract
10	2.303	38.931		10.426	
20	2.996	41.637		12.112	
40	3.689	44.876	77.022	29.015	137.893
60	4.094	49.845		38.975	

The ethanol extract of leilem leaves is still considered to have the potential to inhibit pancreatic lipase enzyme activity but with a higher concentration level than the positive control. Relative potency is a measure of how likely a sample or extract is to suppress pancreatic lipase enzyme activity in a way that is equivalent to a positive control, like orlistat. The results of calculating the relative potential of the inhibitory ability of ethanol extract of leilem leaves of 0.558 support this. The calculation results show that the ethanol extract of leilem leaves can inhibit the activity of pancreatic lipase enzyme with an effective potency of about 55% compared to the positive control drug Orlistat. Therefore, further research is needed to find better methods to maximize the utilization of the potential of Leilem leaf extract in the treatment of obesity and the prevention of diabetes.

### Conclusions

The results of in vitro studies have shown the potential of Leilem leaf extract to inhibit pancreatic lipase enzyme activity. Qualitatively, the results showed that leilem leaf extract contains secondary metabolite compounds such as Alkaloids, Flavonoids, Tannins, Saponins, Steroids, and Terpenoids. Quantitatively, the results showed that the ethanol extract of leilem leaves had an IC<sub>50</sub> value of 137.89 µg/mL, while the IC<sub>50</sub> value of the positive control Orlistat was 77.022 µg/mL. This value corresponds to the concentration of Leilem leaf extract that can reduce enzyme activity by 50%. Based on the IC<sub>50</sub> value, the ability to inhibit the pancreatic lipase

enzyme activity of leilem leaf extract is still below Orlistat as a positive control. The calculation results show that the ethanol extract of leilem leaves can inhibit the activity of pancreatic lipase enzyme with an effective potency of about 55% compared to the positive control drug Orlistat.

This study still needs improvement and refinement of the method. The limited concentration series and inefficient modification methods make the results of this study still very limited and need to be developed. Studies on the utilization of herbal plants in the prevention and treatment of chronic diseases need to be continued. Leilem leaf herbal plants are endemic plants that are still quite rarely researched, so the discovery of their potential and benefits has not been maximized.

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**Informed Consent Statement:** Not applicable.

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**Conflicts of Interest:** The authors declare that there are no conflicts of interest regarding the publication of this manuscript

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