IN VITRO PANCREATIC LIPASE INHIBITOR ACTIVITY OF Mangifera foetida LEAVES EXTRACT

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INTRODUCTION

Obesity is a condition where there is excessive fat in the body. Weight loss can be accomplished by inhibiting fat absorption through the inhibition of pancreatic lipase activity, a key enzyme in fat metabolism. Natural products from plants contain various phytochemical compounds that can act as pancreatic lipase inhibitors. This research was conducted to determine the lipase inhibitor activity of the methanol extract of Mangifera foetida leaves. Mangifera foetida leaves were extracted and then the total phenolic and flavonoid levels were determined. The extract was tested for its ability to inhibit pancreatic lipase in vitro. The inhibitory activities of the extract and Orlistat were measured using p-nitrophenyl palmitate as a substrate at concentrations of 31.25, 62.5, 125, 250, and 500 µg/mL. The results showed that the extracts of young leaves (YL) and mature leaves (ML) contained phenol and flavonoid compounds. The IC50 values of young leaf extract (YL) and mature leaves (ML) were 45.22 and 35.50 µg/mL, respectively. In conclusion, the Mangifera foetida leaf extract can be promoted as a good source of anti-obesity compounds.

Keywords: flavonoid, Mangifera foetida, obesity, pancreatic lipase, total phenolic.
ble for 50-60% of fat absorption (Bauer et al., 2005). Currently, the only clinically available pancreatic lipase inhibitor is Orlistat, which is a derivative of Lipstatin, an irreversible pancreatic lipase inhibitor from Streptomyces (Liu et al., 2020). However, other alternative treatments using active compounds from natural products have been widely studied to obtain a more effective obesity treatment and fewer side effects (Bajes et al., 2020). Natural products from plants have received a lot of attention for their use as anti-obesity. Some research showed that various bioactive compounds from plants can reduce body weight and prevent obesity (Yun, 2010). One of the main phytochemical compounds that play an important role in inhibiting pancreatic lipase activity is polyphenols (Buchholz & Melzig, 2015).

Bacang, *Mangifera foetida*, is a tropical plant commonly found in Indonesia. This plant belongs to the mango family (Anacardiaceae). The fruit is edible but less popular than commercial mangoes (*Mangifera indica*). Moreno et al. (2006) reported that the ethanol extract of the leaves and stem bark of *Mangifera indica* contained polyphenols and can inhibit pancreatic lipase in animal models. Furthermore, research on methanol fruit extracts of seven varieties of *M. indica* has shown that all of them can inhibit pancreatic lipase (Pradhan et al., 2017). Several studies have revealed the presence of phenols, flavonoids, carotenoids, terpenes, terpenoids, and antioxidant activity of *M. foetida* fruits (Tyug et al., 2010; Khoo et al., 2016). However, studies of other parts of the plant, especially leaves, are still lacking. Thus, the objectives of this study were to investigate the polyphenol content including flavonoids, and to assess the lipase inhibitory property of *M. foetida* leaves.

**MATERIALS AND METHODS**

**Preparation of Plant Material**

*Mangifera foetida* leaves were obtained from Temanggung region, Indonesia in August 2019. The leaves were put into two groups based on their position on the twig (from apex to base). Young leaves (YL) are the first 4 leaves at the top. Meanwhile, mature leaves (ML) are the fifth to eighth leaves from the apex. *M. foetida* leaves were washed under running water and dried in an oven at 50°C for 24 hours. Then, the dried leaves were grounded using a mechanical blender to obtain a fine leaf powder. The leaf powder was stored in an airtight container with an appropriate label for further use.

**Preparation of Leaves Extract**

The leaf powder (100 g) was extracted using 96% methanol (500 ml) for 72 hours at room temperature. Afterward, the mixture was filtered using filter paper to get the filtrate from the extraction process. The filtrate was concentrated with a rotary evaporator at 55°C until a thick extract was obtained. Finally, *M. foetida* leaf extract was stored at 4°C for further use.

**Determination of Total Phenol Content**

Total phenolic content (TPC) was measured using the Folin-Ciocalteu test based on the method described by Shoib & Shahid (2015) with modification. The first stage began with preparing a standard solution of gallic acid. Gallic acid (5 mg) was dissolved in methanol to obtain a final concentration of 500 mg/mL. Serial dilutions were then prepared to obtain a concentration of 31.25, 62.5, 125, 250, and 500 µg/mL. An amount of 0.1 mL of each concentration of the stock solution and extract solution (1000 µg/mL) was
added with 7.9 mL of distilled water, 0.5 mL of Folin-Ciocalteau reagent, and 1.5 mL of 20% Na₂CO₃. The reaction mixture was homogenized and incubated for 90 minutes at room temperature. The absorbance was measured using a UV-vis spectrophotometer at a wavelength of 775 nm. The gallic acid calibration curve was made using a linear regression equation (y = a + bx) based on the absorbance value obtained. The TPC determination results were expressed as gallic acid equivalent (GAE)/g of extract. All determination was performed in triplicate.

**Determination of Flavonoid Content**

Flavonoid content (FC) was determined using aluminum chloride colorimetric assay based on the method described by Chandra et al. (2014) with modifications. Quercetin was used as a standard solution. The determination of FC began with the preparation of a quercetin standard curve. Firstly, the stock solution was made by dissolving quercetin (5 mg) in 10 mL methanol. Then, the stock solution was diluted with methanol to obtain serial dilutions (0.015, 0.030, 0.045, 0.060, and 0.075 μg/ml). Meanwhile, the extract solution was prepared by dissolving 5 g of the extract in 100 mL ethanol. An amount of 1 mL of each concentration of the stock solution and extract solution was mixed with 5 mL of 5% AlCl₃ and 4 mL of distilled water. The mixture was homogenized and incubated for 60 minutes at room temperature. The absorbance value was measured using a UV-vis spectrophotometer at a wavelength of 420 nm. The absorbance value was then used to construct a standard quercetin curve to obtain a linear regression equation (y = a + bx). The FC of the sample was calculated from the calibration plot and expressed as mg quercetin equivalent (QE)/g of extract. All tests were performed in triplicate.

**Pancreatic Lipase Inhibition Assay**

The Pancreatic lipase inhibition assay was adopted from Jaradat et al. (2019) with some modification. In this test, p-nitrophenyl palmitate (pNPP) was used as the substrate. The plant extract stock was prepared by dissolving the plant extract in 10% DMSO and Tris HCl buffer (pH 8) to obtain a concentration of 1000 μg/mL. Subsequently, five different concentrations were prepared by serial dilution (5, 12.5, 25, 50, and 100 μg/mL). Porcine pancreatic lipase (10 mg) was dissolved in 10 mL of Tris HCl buffer pH 8. A substrate stock solution (pNPP) was prepared by dissolving 0.19 g of pNPP in 10 mL acetonitrile. The inhibition activity test was carried out by mixing 0.1 mL of lipase (1 mg/mL), 0.2 mL of extract solution (each different concentration) and 0.7 mL of Tris HCl buffer (pH 8). The reaction mixture was homogenized and incubated for 15 minutes at 37°C. After the incubation period, the reaction mixture was added with 0.1 mL of substrate solution (pNPP). Then, the solution was re-incubated for 30 minutes at 37°C. The absorbance was measured using a UV-vis spectrophotometer at a wavelength of 410 nm. Orlistat was used as a positive control. The same procedure was repeated for orlistat using the same concentration as mentioned above. All experiments were carried out in triplicate. Percent inhibition was calculated using the following equation:

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\% \text{ inhibition} = \frac{(A0-A)}{A0} \times 100\%
\]

where A is the activity of the solution with inhibitor and A₀ is the activity of the solution without the inhibitor. The IC₅₀ is the sample concentration required to inhibit enzyme activity by 50%. The IC₅₀ was calculated based on the plot in the regression equation between substrate concentration and percent inhibition results.
RESULTS AND DISCUSSION

Methanolic Extracts of *M. foetida* Leaves

The solvent used for extraction must be selected based on the polarity of the solute. A solvent with the same polarity as the solute will dissolve the solute properly (Altemimi et al., 2017). In this study, *M. foetida* leaves were extracted using the maceration method with methanol as a solvent. Based on previous reports, methanol can dissolve various polar phytochemicals (Houghton & Raman, 1998; Widyawati et al., 2014). Through the extraction process, 28 g of a young leaf (YL) extract and 26 g of mature leaf (ML) extract were obtained. Based on these results, the yield of young leaf extract was 0.28% (w/w) while the yield of mature leaf extract was 0.26% (w/w).

Determination of Total Phenolic Content

Total phenol content (TPC) was expressed as mg gallic acid equivalents (GAE). The determination of TPC in the sample was carried out using the linear equation of the standard curve for gallic acid (Figure 1A). The concentrations of gallic acid used were 31.25, 62.5, 125, 250, and 500 µg/mL. Based on the plot results on the standard curve, the TPC of the YL extract was 136.77 mg GAE/g while the ML extract was 200.77 mg GAE/g. The different TPC results were probably caused by different leaf development stages, wherein in this study the TPC of mature leaf (ML) extract was higher than that of young leaf (YL) extract (Figure 1B). The different stages of leaf development are the main factors that influence the composition and content of bioactive compounds (Chang et al., 2018; Chen et al., 2018). Moreover, the phytochemical content may vary in each plant. It is mainly influenced by environmental conditions during cultivation (Szakiel et al., 2011; Ncube et al., 2012). Various studies have shown that phytochemical content in plants was influenced by several environmental factors such as temperature, humidity, insects, fertilizers, and pesticides (Borges et al., 2013; Liu et al., 2016; Samaniego et al., 2020). In addition, the extraction method can also affect the amount and composition of the bioactive compounds from the extracted sample (Altemimi et al., 2017).

Polyphenols are organic compounds found in various plants. Polyphenols have various health benefits such as antioxidants, anti-inflammatory, and antimicrobial (Buchholz & Melzig, 2015). Polyphenols also have a positive effect on the prevention and treatment of various diseases such as cardiovascular, neurodegenerative disorders, cancer, and obesity (Singh et al., 2011; Cory et al., 2018). Several studies have demonstrated that the subclass of polyphenols such as flavonoids, phenolic acids, and lignans can inhibit pancreatic lipase (Buchholz & Melzig, 2015; Martinez-Gonzalez et al., 2017a). The degree of inhibition is influenced by the number and position of phenol hydroxyl groups, and also by the degree of polymerization and glycosylation elimination (Buchholz & Melzig, 2015).

Determination of Flavonoid Content

In this study, the flavonoid content (FC) was expressed as mg quercetin equivalent (QE). Quercetin standard curves were prepared by measuring the absorbance of the quercetin-AlCl3 complex at concentrations of 0.015, 0.030, 0.045, 0.060, and 0.075 µg/mL at a wavelength of 420 nm (Figure 2A.). The results of FC determination showed that the total flavonoid content in the YL extract was 14.3222 mg.QE/g while the ML extract was 26.4404 mg.QE/g. As seen from the diagram in Figure 2B, the total number of flavonoids in mature leaves was much higher than in young...
leaves. It was probably due to differences in leafage which could lead to different phytochemical profiles.

Flavonoids, a class of secondary metabolites produced by plants which are a group of polyphenols. These compounds are present in all parts of the plant including leaves, roots, flowers, and fruits (Panche et al., 2016). Flavonoids have an important role in plants such as regulating plant development, pigmentation, UV protection, and self-defense (Mathesius, 2018). Flavonoids are well-known compounds that have various biological activities, for example, antioxidants, anti-inflammatory, and antimicrobial (Xie et al., 2015; Panche et al., 2016). Numerous studies have reported the activity of flavonoids as inhibitors of pancreatic lipase such as quercetin (Martinez-Gonzalez et al., 2017a), catechin (Sae-Tan et al., 2011), and kaempferol (Marrelli et al., 2018). Compared to other polyphenols (phenolic acids), the higher inhibitory activity of flavonoids appears to be associated with the structural complexity of flavonoids when interacting with enzymes (Martinez-Gonzalez et al., 2017b).

In this study, the phenolic and flavonoid content was higher in the mature leaves and lower in the young leaves. The difference in leaf age might explain this finding. Variations in phenolic and flavonoid levels are caused by different ages of leaf development (Anwar et al., 2017). The position of the leaves on the twig shows their age where the leaves at the apex are the youngest. In young leaves, secondary metabolite compounds are still not produced in large quantities. Meanwhile, in mature leaves, secondary metabolites have accumulated so that the phenolic and flavonoid levels are higher. However, in the older leaves, the secondary metabolite levels generally decrease. In addition, levels of secondary metabolite compounds in leaves are also influenced by environmental conditions.

**Pancreatic Lipase Inhibitory Effect**

In this study, extracts of young leaves (YL) and mature leaves (ML) of *M. foetida* were assessed for their activity as an inhibitor of porcine pancreatic lipase. Pancreatic lipase is synthesized and secreted by the pancreas. This enzyme was responsible for the digestion of lipids in the body. Currently, antilipase activity is the most studied mechanism in determining the efficacy of natural products as anti-obesity agents.

The inhibitory activity of *M. foetida* leaf extract against pancreatic lipase is presented in Figure 3. Inhibition rates of positive control (Orlistat) and plant extracts depend on the concentration used. The inhibition of enzyme activity has increased as the concentration of *M. foetida* leaf extract increased (Figure 3). *M. foetida* leaf extracts significantly inhibited porcine pancreatic lipase activity using p-nitrophenyl palmitate-based assay. At 50 µg mL⁻¹, the inhibitory activity of the YL and ML extracts was 55% and 72%, respectively.

The IC50 value was calculated by plotting the inhibition curve of pancreatic lipase. The IC50 value is the half-maximum inhibitory concentration or inhibitor concentration that can inhibit lipase activity by up to 50%. This value is generally used to express the inhibitory effect of an inhibitor compound on lipase. The IC50 values of the YL and ML extract were 45.22 and 35.50 µg/mL, respectively.
Figure 1. (A) Standard calibration curve of Gallic acid (B) Total phenolic content of *Mangifera foetida* extract. Young leaves (YL) and mature leaves (ML). Data were presented as mean ± SD (n = 3).

Figure 2. (A) Standard calibration curve of Quercetin (B) Flavonoid content of *Mangifera foetida* extract. Young leaves (YL) and mature leaves (ML). Data were presented as mean ± SD (n = 3).

Figure 3. Porcine pancreatic lipase inhibitory activity of young leaves (YL), mature leaves (ML) of *Mangifera foetida* extract and Orlistat. Data were presented as mean ± SD (n = 3).
As shown in Figure 3, both YL and ML extract can inhibit pancreatic lipase. Compared to YL extract, ML extract had a lower IC50 value, which means that ML extract was better at inhibiting porcine pancreatic lipase. In addition, the IC50 value of *M. foetida* leaf extract was compared to the standard lipase inhibitor, Orlistat, which had an IC50 value of 22 µg/mL. In this study, Orlistat was more potent than *M. foetida* leaf extract probably because this extract was still a crude extract which may consist of various compounds, both active and inactive components. The results of this in-vitro study confirm the potential activity of *M. foetida* leaf extract as an anti-lipase agent.

The difference in composition and amount of secondary metabolite compounds affects pancreatic lipase activity between young and mature leaves. The inhibitory ability might be directly related to the higher content of polyphenols and flavonoids in mature leaves. According to Dechakhamphu & Wongchum (2015), phenolic and flavonoid contents have a positive correlation with their inhibitory activity against pancreatic lipase. Therefore, these phytochemicals are probably one of the key agents for pancreatic lipase inhibition. In addition, flavonoids are also known as powerful antioxidants to combat oxidative stress. Oxidative stress is a contributing factor in the development of obesity and its associated complications. Therefore, flavonoids might be able to provide multiple benefits, which inhibit pancreatic lipase as well as oxidative stress (Jo et al., 2017).

Mangiferin is natural plant polyphenols that belong to the xanthonoid group. Mangiferin has been extensively reviewed for its extraordinary health-related properties such as antiviral, anticancer, antidiabetic, antioxidant, and UV protection (Imran et al., 2017; Matkowski et al., 2013; Khurana et al., 2016; Du et al., 2018). Itoh et al. (2016) have reported that mangiferin is one of the bioactive compounds found on the leaves of *Mangifera indica* cv Irwin which can inhibit pancreatic lipase. This active compound can be found in various higher plants and almost in all parts of *M. indica*, such as bark, stems, leaves, fruits, and roots. According to Barreto et al. (2008), the highest mangiferin levels in *M. indica* are on the leaves. Soediro et al. (1991) has compared seven cultivars of mango in Indonesia and found that *M. foetida* leaves had the highest mangiferin content. In silico studies confirm mangiferin inhibits human pancreatic lipase by allosteric binding which

![Figure 4. The half-maximal inhibitory concentration (IC50) of young leaves (YL), mature leaves (ML) of *Mangifera foetida* extract and Orlistat, data were presented as mean ± SD (n = 3).](image-url)
forms hydrogen bonding with Lys238, Cys261, and Asp205 (Picot et al., 2017). However, it is also possible that inhibition of pancreatic lipase might result from the synergistic interaction of the various components present in M. foetida leaf extract.

Plants have a variety of beneficial bioactive compounds that can accumulate in plants. Various studies have proven that plants are rich in phytochemicals. Thus, the consumption of natural products derived from plants is believed to improve health. In several Southeast Asian countries, young Mangifera indica leaves are commonly eaten fresh as a salad or side dish (Cahyanto et al., 2020). This habit has become a local tradition. However, studies on the potential of Mangifera members, such as M. foetida are still limited. To the best of the author's knowledge, there is no previous research on the effect of M. foetida leaf extract on pancreatic lipase. Data obtained from this study suggest that M. foetida leaf extract has an inhibitory effect on pancreatic lipase. Further in vivo studies are needed to confirm these findings. In addition, other studies are also needed to identify the bioactive compounds in these plants that are responsible for the inhibition of pancreatic lipase.

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