



Lipase Enzyme Inhibitory Activity of Jombang Leaves Extract (*Taraxacum officinale* F.H. Wigg)

(Aktivitas Penghambatan Enzim Lipase Ekstrak Daun Jombang (*Taraxacum officinale* F.H. Wigg) Secara In Vitro)

Risa Adia, Annisa Farida Muti*, Via Rifkia, Dhigna Luthfiyani Citra Pradana

Jurusan Farmasi, Fakultas Kedokteran, Universitas Pembangunan Nasional “Veteran” Jakarta, Jakarta, Indonesia.

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Corresponding Author:

Annisa Farida Muti

Program Studi Farmasi

Fakultas Kedokteran

Universitas Pembangunan Nasional

“Veteran” Jakarta

Jakarta

12450

Indonesia

email: afmuti@upnvj.ac.id

ABSTRACT

Background: Obesity is a condition that occurs due to an imbalance between energy intake and expenditure. Lipase enzymes play an important role in the process of fat metabolism, making it a target in obesity treatment strategies. Jombang plant has been used for generations as an alternative treatment for various diseases. The main content of secondary metabolite compounds in jombang plants are phenolics, flavonoids, terpenoids, and alkaloids. **Objective:** This study was conducted to determine the activity and potential inhibition of jombang plant (*Taraxacum officinale* F.H. Wigg.) against lipase enzyme *in vitro*. **Materials and Methods:** The phytochemical screening was determined by a color change reaction with certain reagents, while total phenolic content was carried out using Folin-Ciocalteu reagent spectrophotometrically. The inhibitory activity of 96% ethanol extract of jombang leaves as well as orlistat as positive control was measured by titration method and using olive oil substrate. The extract concentrations used were 125 ppm, 250 ppm, and 500 ppm. **Results:** Jombang leaf extract contains alkaloid, phenolic, flavonoid and terpenoid compounds qualitatively. Determination of total phenolic content in 96% ethanol extract of jombang leaves was obtained at 12.69 ± 0.91 mgGAE/g. The highest percentage of inhibition was found at a concentration of 500 ppm which amounted to 103.85%. The difference in the percentage of inhibition in all groups showed no significant difference (Sig>0.05). **Conclusion:** This study shows that jombang leaf extract is able to inhibit lipase enzyme activity with the highest percentage of inhibition was 103,85%.



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INTRODUCTION

Obesity is a condition that occurs when there is an increase in energy storage due to an imbalance between energy intake and expenditure, causing hyperplasia and hypertrophy of adipocytes. Genetic and environmental factors greatly contribute to the development of obesity (DiPiro, 2020). The liver, muscles, and pancreas are typical non-adipose tissue organs in which adipocyte hypertrophy has been shown to increase levels of free fatty acids and lipotoxicity. Adipocyte hypertrophy may also impact the process of storing fatty acids (Vekic et al., 2019).

An individual is considered overweight if their body mass index falls between 25.0 and 29.9 kg/m², and they are considered obese if it exceeds 30 kg/m² (Lin & Li, 2021). Risk factors that are commonly found in the obese category include hypertension, hyperlipidemia, coronary heart disease, insulin resistance, glucose intolerance, and diabetes (DiPiro, 2020). Every year, there are more cases of obesity. According to data from Riset Kesehatan Dasar (2018), obesity cases among Indonesians over the age of 18 increased from 14.8% to 21.8% (Kementerian Kesehatan RI, 2018).

Triacylglycerol lipase (EC 3.1.1.3) or lipase enzyme plays a role in the metabolism of fats. This enzyme will catalyze the hydrolysis of fats and oil into fatty acids and glycerol. The main substrate of lipase enzyme is triacylglycerol (Jaiswal et al., 2017). The lipase enzyme has an important role in fat metabolism, so inhibition of this enzyme is also one of the strategies in treatment (Kim et al., 2020).

Orlistat (Tetrahydrolipstatin) is the only anti-obesity that reduces calorie intake by inhibiting the lipase enzymes in the stomach and pancreas (Gadde et al., 2018). Orlistat should be taken three times a day at a dose of 120 mg, one hour after a meal containing fat (DiPiro, 2020). Side effects that can occur include gastrointestinal issues such as bloating, greasy stools, and fecal incontinence (Gadde et al., 2018).

For many years, Indonesians have employed jamu and medicinal herbs as alternative treatments. The consumption of herbs and medicinal herbs tends to grow over time (Satria, 2013). *Taraxacum officinale* F.H. Wigg, commonly called jombang plants, is one of the plants used as an alternative medicine and is commonly found since it grows wild (Badrunasar dan Harry, 2017). The secondary metabolites contained in the jombang herb are flavonoids, phenolics, steroids, triterpenes, and amino acids (Jedrejek et al., 2019). *Chicoric acid*, *chlorogenic acid*, and *caffeic acid* are the primary phenolic compounds present in the Jombang plants, whereas *lutein-7-glucoside*, *apigenin-7-rutinoside*, and *apigenin-7-glucoside* are the main flavonoid components (Di Napoli & Zucchetti, 2021; Grauso et al., 2019). Several studies have shown that the jombang leaves have pharmacological activities such as anti-obesity, hepatoprotective, antidiabetic, antioxidant, antimicrobial, and anti-inflammatory (Aabideen et al., 2020; Choi et al., 2018; Herrera-pool et al., 2021; Pfingstgraf et al., 2021; Xie et al., 2018; Xue et al., 2017).

It is believed that phenolic and flavonoid components in jombang leaves are essential to inhibiting the lipase enzyme from working properly. Previous research showed that the total phenolic content in the 96% ethanol extract of jombang leaves was 8.00 mg GAE/g (Ivanov, 2014). Zhang et al.'s (2008) research on the lipase enzyme inhibitory ability showed that luteolin, a flavonoid present in a 95% ethanol extract of jombang leaves, was able to effectively block lipase enzymes by 86.3% (J. Zhang et al., 2008).

Based on this description, the researcher expressed interest in experimenting to evaluate the lipase enzyme inhibitory activity of 96% ethanol extracts of jombang leaves (*Taraxacum officinale* F.H. Wigg) *in vitro* with different methods.

MATERIAL AND METHODS

Materials

Jombang leaves (*Taraxacum officinale* F.H. Wigg) were obtained from Balai Besar Pengembangan dan Penelitian Tanaman Obat dan Obat Tradisional (B2P2TOOT), Potassium dihydrogen phosphate (Pudak Scientific), orlistat 120 mg (NOVELL), Lipase from Human Pancreas E.C. 3.1.1.3, ethanol 96%, Olive oil, Gum Arabic, mayer's reagent, methanol, magnesium, HCl, NaOH, distilled water, Na₂CO₃, FeCl₃, H₂SO₄, chloroform, NaNO₂, Gallic Acid, folin-ciocalteu reagent 10%, KI, iodine, CaCl₂, and HgCl₂ that purchased from Sigma Aldrich.

Methods

Extraction of Jombang Leaves

The maceration method was used to extract the jombang leaves simplicia., which refers to the preparation of extracts in the Farmakope Herbal Indonesia (Kementerian Kesehatan RI, 2017), with a few modifications. The simplicial powder that has been obtained is weighed to as much as 200 grams and then extracted using 2 L of 96% ethanol in a macerator for 4 x 24 hours with occasional stirring. Then it was filtered with filter paper and evaporated using a vacuum rotary evaporator at a temperature of 50°C until a thick extract is obtained and weighed.

Screening for Phytochemical Compounds

Alkaloids

Mayer's and Wagner's assays were used to identify these alkaloids. A few drops of Mayer's reagent were added to the extract, and color change was observed. Alkaloids are present when a precipitate that is yellow or white forms. A few drops of the reagent are added to the extract to perform the Wagner test. Alkaloids are indicated by a brown precipitate (Farnsworth, 1966; Iqbal et al., 2015; Kancherla et al., 2019; Mandal et al., 2015).

Flavonoids

As much as 0.5 g of extract was put into a test tube, 5 mL of methanol was added, and the tube was heated over a water bath for 5 minutes. Then a small amount of magnesium powder and a few drops of HCl are added. The color that ranges from orange to dark red indicates the presence of flavonoids (Farnsworth, 1966; Parbuntari et al., 2019).

Saponins

A test tube containing up to 0.5 g of the extract was filled with 10 mL of distilled water, and the tube was heated and shaken. The formation of foam, which persisted for 10 minutes, indicated the presence of saponins (Farnsworth, 1966; Parbuntari et al., 2019).

Phenolic and tannins

As much as 0.5 g of extract was put into a test tube, 10 mL of distilled water was added, and the mixture was stirred. Then add a few drops of the FeCl₃ solution. The presence of tannins is indicated by a change in color or by a blue, green, blackish-blue, or blue-green precipitate, whereas the presence of phenolic compounds is indicated by a dark green color (Farnsworth, 1966; Parbuntari et al., 2019).

Steroids or Terpenoids

As much as 100 mg of the extract was put into a test tube, and then the Lieberman-Bouchard reagent was added. Then 2 mL of H₂SO₄ was added through the test tube wall. Steroids are present when there is the production of a brown ring between the two layers when the top layer turns green, and triterpenoids are present when there is the formation of a dark red hue (Farnsworth, 1966; Parbuntari et al., 2019).

Determination of Total Phenolic Content

The determination of total phenolic content was carried out using gallic acid is used as a standard solution, based on Sukweenadhi et al., (2020). because it is classified as a natural and stable phenol and is relatively inexpensive compared to the others (Aswad et al., 2021). Gallic acid was employed at concentrations of 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm. 10 mL of distilled water were used to dissolve up to 10 milligrams of jombang leaves extract. Then, 0.5 mL of a standard gallic acid solution or extract was reacted with 0.5 mL of folin-ciocalteau reagent (1:1), shaken until homogeneous for one minute, and incubated. Before the eighth minute of incubation, 4 mL of 7.5% Na₂CO₃ and distilled water was added, up to 10 mL. The solution was measured at a wavelength of 760 nm using a UV-Vis spectrophotometer. For replication, the test was run three times.

In vitro Lipase Enzyme Inhibition Activity Testing

Testing the inhibitory activity of the lipase enzyme was carried out based on Subandi et al., (2019) study, using the titration method with modifications. To prepare the substrate, 2.5 mL of olive oil was mixed with 22.5 mL of a 10% (w/v) gum arabic solution, and the mixture was homogenized for 10 minutes. After that, 20 mL of water, 15 mL of 0.075 M CaCl₂, and 10 mL of 3 M NaCl were added and homogenized again. The solution was added with phosphate buffer (pH 7.4) to 100 mL, then homogenized. Variations in the concentration of the extract solution used were 125, 250, and 500 ppm.

The erlenmeyer flask was filled with a test sample (120 mg of orlistat or extract), 25 mL of substrate solution, 1 mL of lipase enzyme, and it was homogenized for 30 seconds. The solution was then incubated at 37°C for 25 minutes. Following incubation, the solution was heated for 10 minutes at 100°C over a water bath. The titration process uses 0.1 N NaOH solution and Phenolphthalein as an indicator. When a color change to pink occurred, the titration was terminated. Equation (1) was utilized to determine lipase activity.

$$\text{Lipase activity } (\mu\text{Mol}/\text{min}) = \frac{(V_{sm} - V_{sa}) \times N_{NaOH} \times 1000}{25} \dots\dots\dots(1)$$

- V_{sm} = The volume of NaOH required to titrate the oil substrate
- V_{sa} = The volume of NaOH required to titrate the water substrate
- N_{NaOH} = Normality of NaOH
- 1000 = Conversion factor from mMol to μMol
- 25 = incubation time (minute)

The percentage of inhibitory activity was calculated using the following equation (2).

$$\% \text{ Inhibition} = \frac{A_1 - A_2}{A_1} \times 100 \dots\dots\dots(2)$$

A₁ = Lipase activity without inhibitor; A₂ = Lipase Activity with inhibitor (orlistat/sample)

RESULTS AND DISCUSSION

Screening for Phytochemical Compounds

Phytochemical screening was carried out to determine the content of secondary metabolites qualitatively by using a color change reaction (Hayat et al., 2020). The results of the phytochemical screening of the jombang leaves extracts contain alkaloids, phenolics, flavonoids, and terpenoids which are listed in Table 1. According to several studies, jombang leaves include phenolic compounds, tannins, flavonoids, terpenoids, and alkaloids (Edori & Marcus, 2019; Jedrejek et al., 2019).

Table 1. Results of Phytochemical Screening of Jombang Leaves Extract

Test type	Sample
Alkaloids	+
Flavonoids	+
Saponins	-
Tannins/Phenolics	+
Steroids/Terpenoids	+

(+) contain secondary metabolites; (-) not contain secondary metabolites

The phytochemical screening of alkaloid compounds was carried out using Mayer's and Wagner's reagents. When Mayer's reagent (mercury (II) chloride) is added, the jombang leaves extract forms a white precipitate, which is proof that it contains an alkaloid component. The potassium-alkaloid complex is what causes the white precipitate to develop when the nitrogen groups in the alkaloids react with the K^+ metal ion from potassium tetraiodomercurate (II) (Parbuntari et al., 2019). Additionally, Wagner's reagent was added to the alkaloid test, which revealed the presence of alkaloids by producing a brown precipitate. Iodine and I^- ions from potassium iodide react with Wagner's reagent, resulting in the formation of brown I_3^- ions. The brown precipitate seen in jombang leaves extract is an alkaloid-complex precipitate produced by covalent interactions between alkaloid nitrogen groups and K^+ metal ions (Setyowati et al., 2014).

The results of screening for phenolic compounds using $FeCl_3$ solution showed that the jombang leaves extract contained phenolic compounds, which were indicated by the formation of a black-green solution. Phenolic compounds most commonly found in plants have an aromatic ring with one or more hydroxyl groups (Zhang et al., 2022). The color change of the solution in the jombang leaves extract occurs due to the formation of an iron (III) hexaphenolate complex between the hydroxy groups and Fe^{3+} ions. Additionally, flavonoid compounds are also contained in jombang leaves extract. Flavonoids in the extract will be reduced by magnesium and hydrochloric acid which causes the formation of a red hue (Habibi et al., 2018).

The saponin test showed that the jombang leaves extract did not contain saponins, as indicated by the absence of froth after shaking. Saponin compounds have polar groups in the form of glycosyl and steroid groups and terpenoids as nonpolar groups. Saponins will form micelles when they are shaken with water, with the polar group facing outward and the nonpolar group facing inside (Parbuntari et al., 2019).

The appearance of brown rings during a steroid or terpenoid test using the Liebermann-Buchard reagent indicated that the jombang leaves extract contained terpenoids. Terpenoids are isoprene-based secondary metabolite molecules (Déclaire Mabou et al., 2021). The terpenoid compounds found in jombang leaves are sesquiterpene lactones (taraxinic acid, taraxacoside) and triterpenes (Di Napoli &

Zucchetti, 2021). The brown ring in the jombang leaves extract is formed from the reaction between H_2SO_4 and acetic anhydrous (Habibi et al., 2018).

Determination of Total Phenolic Content

The total phenolic content of the jombang leaves extract was determined using the gallic acid standard curve. From the results of measuring the absorbance and concentration of the gallic acid standard solution, a standard gallic acid standard curve was obtained with the equation $y = 0.0071x + 0.0865$ and a correlation coefficient (R) of 0.9992 (Figure 1).

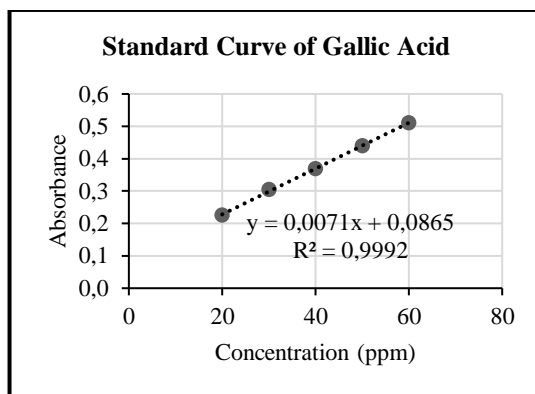


Figure 1. Standard Curve of Gallic Acid

Table 2. Total Phenolic Content of Jombang Leaves Extract

Sample	Total Phenolic Content (mgGAE/g)
96% ethanol extract	12,69±0,91

Determination of total phenolic content in 96% ethanol extract of jombang leaves was obtained by $12,69 \pm 0,91$ mgGAE/g (table 3). According to earlier studies Xue et al., (2017), the total phenolic content of jombang leaves extracted with 80% ethanol, was 23.27 ± 1.43 mgGAE/g. Phenolics are important bioactive compounds in plants because they can reduce reactive oxygen species in the body, stimulate energy expenditure, increase lipolysis, activate β -oxidation processes, and improve lipid metabolism disorders (Boccellino & D'Angelo, 2020).

In Vitro Lipase Enzyme Inhibition Activity Testing

The titration method was used to measure the inhibitory activity of the lipase enzyme. The principle of the titration method is based on the release of fatty acids from triacylglycerols through a hydrolysis process catalyzed by lipase. This method involves the incubation of the sample and an endpoint for the base titration of the acid released. The results obtained depend on the lipase activity. The titrant commonly used is NaOH. The substrate recommended for use in the lipase activity test is olive oil emulsified in gum Arabic (Stoytcheva et al., 2012). Lipase activity in this method is also based on the

amount of NaOH titrant, where the smaller the amount of titrant, the smaller the resulting lipase activity, indicating inhibitory activity (Subandi et al., 2019).

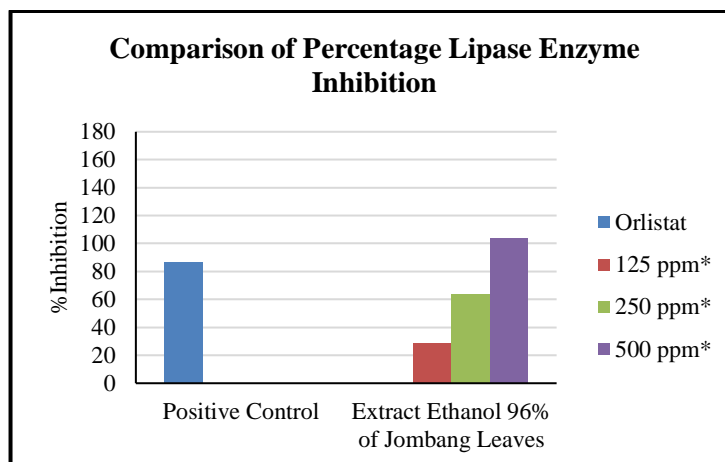


Figure 2. Comparison of Percentage Lipase Enzyme Inhibition (*Sig>0,05 compared to Positive Control)

Figure 2 shows the results of testing the inhibition of jombang leaves extract against the lipase enzyme, the higher the concentration utilized, the greater the percentage of inhibition generated (Subandi et al., 2019). The higher the percentage of inhibition, the more effective the extract is at reducing the activity of the lipase enzyme. A decrease in the activity of the lipase enzyme assumes that the amount of fatty acids released is small due to the inhibition of the enzyme's action. The greatest percentage of inhibition was found in the 96% ethanol extract of jombang leaves at a concentration of 500 ppm, which was 103.85%. The test results obtained were higher than previous studies, namely that the 95% ethanol extract of Jombang leaves had an inhibition percentage of 86.3% at a concentration of 250 ppm (Zhang et al., 2008). This is possible as a result of the various test procedures and concentrations. Additionally, it was discovered that the jombang leaves extract had a greater percentage of inhibition (86.54%) than the positive control (orlistat). According to earlier research using the titration approach, orlistat inhibited lipase enzymes to an extent of 86.96% (Subandi et al., 2019).

Based on the one-way ANOVA statistical test, the value of Sig>0.05 showed that there was no difference in inhibitory activity in all treatment groups, which means that the jombang leaves extract has an inhibitory activity comparable to that of the positive control (orlistat). The high percentage of enzyme inhibition by the 96% ethanol extract of jombang leaves is also influenced by its compound content. The inhibitory action of jombang leaves extract against lipase enzymes is correlated with the concentration of phenolic compounds, with a high content of phenolic compounds likewise translating to a high inhibitory activity (Aabideen et al., 2020).

The main phenolic compounds in jombang leaves are chicoric acid, caffeic acid, and chlorogenic acid (Grauso et al., 2019; Pfingstgraf et al., 2021). Through the AMPK signaling pathway, chicoric acid plays

a role in the process of repairing oxidative stress and inflammation (Ding et al., 2020), whereas chlorogenic acid can induce white adipocytes' browning, which can increase fat oxidation and decrease fat storage (Sudhakar et al., 2020).

Flavonoids belonging to phenolic compounds also play an important role in reducing ROS, capturing radical compounds, reducing fat absorption, suppressing lipid oxidation and stimulating energy expenditure (Aabideen et al., 2020; Pastor-Villaescusa et al., 2018). Another way that flavonoids work is by their ability to interact with digestive enzymes, which makes them able to prevent lipase enzymes from catabolizing fat (Pastor-Villaescusa et al., 2018). Flavonoids can be a method for managing weight reduction due to this mechanism. Terpenoid compounds can competitively inhibit lipase enzymes by modulating PPAR, which can reduce VLDL (Very Low-Density Lipoprotein) production, increase triglycerides catabolism, and promote the formation of HDL (High-Density Lipoprotein) particles (Bougarne et al., 2018; Saad et al., 2021). Alkaloids are also able to influence the process of lipid metabolism by increasing the processes of lipolysis and thermogenesis, which can reduce appetite in obese patients (Saad et al., 2021).

CONCLUSION

According to the study's results, alkaloids, phenolics, flavonoids, and terpenoids, which are secondary metabolites, are present in jombang leaves extract (*Taraxacum officinale* F.H. Wigg). The most significant impact in preventing lipase activity is assumed to be played by phenol and flavonoid chemicals found in jombang leaves. The total phenolic content in a 96% ethanol extract was obtained at $12,69 \pm 0,91$ mgGAE/g. The jombang leaves extract had activity inhibition comparable to the positive control, as shown by the highest percentages of lipase inhibition for the 96% extracts of jombang leaves (*Taraxacum officinale* F.H. Wigg) at a concentration of 500 ppm (Sig>0.05) was found to be 103.85%.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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