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Aktivitas Anti-Lipase Ekstrak Daun Kemuning (Murraya paniculata) dan Fraksi-Fraksinya

Anti-lipase Activity of Kemuning (Murraya paniculata) Leaves Extract and Its Fractions

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Abstrak

Kemuning (Murraya paniculata) termasuk dalam famili Rutaceae, banyak tumbuh di daerah tropis seperti Indonesia. Secara tradisional, daun kemuning digunakan untuk menurunkan berat badan. Daun kemuning mengandung senyawa yang dapat menghambat lipase pankreas, yaitu flavonoid. Penelitian ini bertujuan untuk mengetahui aktivitas antilipase ekstrak daun kemuning dan fraksinya. Ekstraksi daun kemuning dilakukan secara maserasi dengan pelarut etanol 70%. Pemisahan metabolit sekunder dalam ekstrak dilakukan dengan metode kromatografi cair vakum dengan fase diam silika gel dan campuran eluen n-heksana, etil asetat, dan metanol secara gradien. Sedangkan aktivitas antilipase diuji dengan metode kolorimetri dengan p-nitrofenil butirat sebagai substrat dan orlistat sebagai kontrol positif. Ekstrak yang diperoleh sebesar 19,94% dari berat simplisia. Ekstrak yang telah dipisahkan ditampung dalam 20 vial kemudian digabungkan berdasarkan nilai Rf bercak yang diamati pada kromatografi lapis tipis (KLT) menghasilkan 8 fraksi (fraksi A-H). Persentase hasil fraksi A-H berturut-turut sebesar 1,20; 1,33; 1,80; 3,92; 3,57; 4,20; 16,85; dan 14,47%. Aktivitas antilipase ditunjukkan dengan nilai IC_{50} masing-masing sebesar 17,603 ± 1,660 dan 108,554 ± 1,660 µg/mL untuk orlistat dan ekstrak. Aktivitas antilipase fraksi A-H berturut-turut sebesar 130,19 ± 2,90, 119,52 ± 1,95, 96,95 ± 1,81, 91,65 ± 0,78, 95,30 ± 1,46, 114,76 ± 2,87, 127,07 ± 3,56, dan 134,04 ± 3,16 µg/mL. Berdasarkan hasil tersebut dapat disimpulkan bahwa ekstrak daun kemuning dan fraksi-fraksinya mempunyai aktivitas antilipase yang sangat kuat.

***Kata Kunci**: Murraya paniculata; ekstrak; fraksi; anti-lipase*

Abstract

Kemuning (Murraya paniculata) belongs to the Rutaceae family, it grows a lot in tropical areas such as Indonesia. Traditionally, Kemuning leaves are used for weight loss remedies and are believed to contain compounds that can inhibit pancreatic lipase, including flavonoids. This study aimed to reveal the antilipase activity of Kemuning leaves extract and its fractions. The extraction of Kemuning leaves was carried out by maceration with 70% ethanol as solvent. The vacuum liquid chromatography method was used to separate the secondary metabolites in the extract with the stationary phase of silica gel and the eluent mixture of n-*hexane, ethyl acetate, and methanol in a gradient manner. Meanwhile, the antilipase activity was tested by colorimetric method,* with p-*nitrophenyl butyrate as a substrate and orlistat as a positive control. The extract obtained was 19.94% of the weight of the simplicia. The separated extract was collected in 20 flasks and then combined based on the Rf value of the spots observed on thin layer chromatography (TLC) producing 8 fractions (fractions A-H). The percentage yields of fractions A-H were 1.20, 1.33, 1.80, 3.92, 3.57, 4.20, 16.85, and 14.47%, respectively. Antilipase activity was indicated by IC_{50} values, which were 17.603 ± 1.660 and 108.554 ± 1.660 µg/mL for orlistat and extract, respectively. The antilipase activities of fractions*

A-H were 130.19 ± 2.90, 119.52 ± 1.95, 96.95 ± 1.81, 91.65 ± 0.78, 95.30 ± 1.46, 114.76 ± 2.87, 127.07 ± 3.56, and 134,04 ± 3.16 µg/mL, respectively. Based on these results, it can be concluded that the extract and its fractions exhibit very strong antilipase activity.

***Keywords**: Murraya paniculata; extract; fraction; antilipase*

Introduction

Obesity is the accumulation of excess fat in the body because of eating more than the energy spent (Rismawati *et al*., 2012). Obesity can lead to various effects of diseases such as hypertension, heart disease, cancer, and diabetes. Consequently, obesity-related morbidity and mortality will be high and will also affect health costs (Masrul, 2018). Several chemical medications have been developed to prevent obesity, including orlistat (Schwinghammer *et al*., 2021). In the gastrointestinal system, orlistat binds to lipase, preventing the breakdown of dietary lipids. The consequence will be a reduction in the formation of micelles, so that lipids are not absorbed.

Currently, some Indonesian people use medicinal plants to prevent and treat disease, such as obesity. *Kemuning* (*Murraya paniculata*), a plant in the Rutaceae family, is one of the useful medicinal plants (Figure 1). According to traditional use, the *Kemuning* leaves is the most used medication (Amanda *et al*., 2019). The decoction of m. paniculate leaves has been widely used as traditional medicine in Indonesia, particularly for weight loss, treatment of irregular menstruation, vaginal discharge, and rheumatism (Dosoky, *et al*., 2016). Some research show that *Kemuning* leaves have pharmacological activities such as anti-obesity, antioxidant, antidepressant, antianxiety, analgesic, anti-diabetic and antibacterial (Rohman and Riyanto, 2005; Gautam *et al*., 2012b; Rehman *et al*., 2014; Sharma *et al*., 2017).

The medicinal properties of *Kemuning* leaves are based on their chemical composition. *Kemuning* leaves contain secondary metabolites of indole alkaloids, coumarins, flavonoids, saponins, and tannins (Ng *et al.*, 2012). According to a study by Iswantini *et al*. (2011), *Kemuning* leaves inhibit pancreatic lipase activity.

In this study, the lipase inhibitory activity of the Kemuning leaves extract and its fractions were investigated. The purpose of the fraction's investigation is to discover fractions that have anti-lipase activity containing compounds classified as non-polar, semi-polar or polar.

Methods

Kemuning leaves were obtained from Rowosari Village, Sumberjambe District, Jember Regency, East Java Province on October 20, 2020. Ethyl acetate, n-hexane, methanol p.a. (Fulltime), p-nitrophenyl butyrate (Sigma-N9876), tris (hydroxymethyl aminomethane) (Sigma-252859), 1.0 N HCL, DMSO, acetonitrile, distilled water, orlistat (Sigma), and porcine pancreas lipase (Sigma-Aldrich) were utilized as the materials. The instruments included a rotary evaporator (Heidolph), an ultrasonicator (Elmasone), an UV-Vis spectrophotometer (Hitachi U-1800), and a microplate reader (Biotex ELx 800).

The first step in extraction was removing the fat from 150 g of simplicia by soaking it in 750 mL of n-hexane for 24 hours, then filtering it and drying the residue. The dried residue was sonicated for 30 minutes in 750 mL of 70% ethanol before being macerated for 24 hours, then filtered. The filtrate was concentrated using a rotary evaporator to produce extract (Dillasamola *et al*., 2015) and stored in the refrigerator until utilized in the following experiment.

The 3.12-gram extract was loaded into a chromatographic column packed with 60 GF_{254} silica gel and eluted with an eluent of a 10% gradient mixture of n-hexane-ethyl acetate and ethyl acetate-methanol (Sulastry and Kurniawati, 2010), each as much as 80 mL. After the fractionation results were spotted on the TLC plate and eluted with ethyl acetate, the chromatogram profile was analyzed. Fractions having the same chromatogram profile were combined.

The anti-lipase activity of the extract and its fraction was determined by colorimetric assay, in which the release of p-nitrophenol from p-nitrophenyl butyrate was measured according to the previously described method (Lewis and Liu, 2012). Pancreatic lipase solution was prepared immediately before use. Pancreatic lipase was suspended in Tris-HCl buffer (2.5 mM, pH 7.4, containing 2.5 mM NaCl) and stirred for one minute to a concentration of 5 mg/mL (200 units/mL). The suspension was then centrifuged at 6000 rpm for 10 minutes, to obtain the supernatant in the form of a clear solution.

Figure 1.*Kemuning* leaves

A total of 25 µL of pancreatic lipase solution was preincubated with 20 µL of the extract or its fraction (final concentrations 5; 10; 25; 50 and 100 µg/mL) or orlistat is (final concentrations 1; 5; 25; and 50 µg/mL) for 15 minutes at 37 °C in a 96-well microplate. Then 20 µL of the p-NPB substrate (10 mM in acetonitrile) was added. The mixture was diluted to 200 µL with Tris-HCl solution and incubated for 5 minutes before being measured at 410 nm with a microplate reader (Dialab ELx800, Austria). Three replications of the tests were performed. The following equation can be used to calculate the percentage of lipase inhibition.

$$\text{Lipase inhibition (\%)} = \frac{\text{(A blank - A sample)}}{\text{A blank}} \times 100\%$$

Results

Extraction

In this study, the percentage yield of the extract was 19.9% (Table 1).

Fractionation

Extract was loaded into normal phase silica column chromatography, then developed with gradient solvents (n-hexane-ethyl acetate and ethyl acetate-methanol, each as much as 80 mL) to produce 21 fractions. These fractions were then analyzed by TLC, in which fractions with similar profiles were combined.

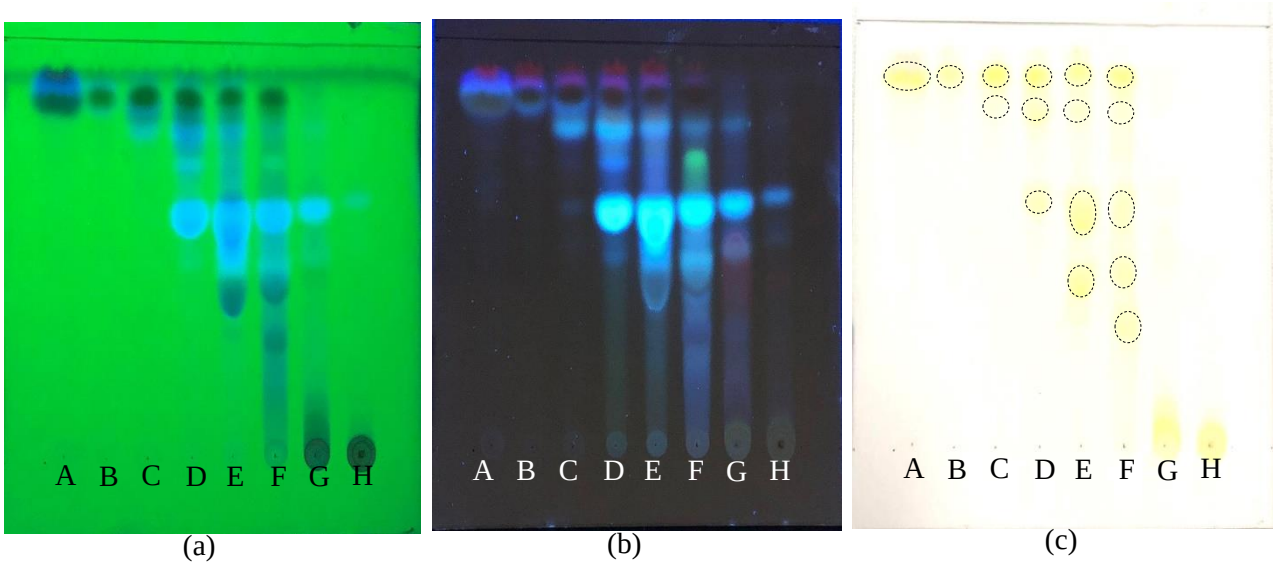
Based on the similarity of the TLC profile, 8 fractions (A–H) were produced, as presented in Figure 1. Figure 1a shows the TLC fractions observed under UV light at wavelength 254 nm, Figure 1b observes under UV light at wavelength 365 nm, and Figure 1c shows the TLC fractions after spraying TLC with citrate-borate. The yield of each fraction is reported in Table 2. A citrate-borate reagent was used to detect the presence of flavonoids. According to the TLC profile, flavonoids were present in the A-F fraction, as illustrated in Figure 2.

Table 1. Extraction results of *Kemuning* leaves

Simplicia weight (g)	Extract weight (g)	Yield (%)
150	29.9	19.9

Table 2. Fractions of *Kemuning* leaves extract

Fractions	flask	Yield of fractions (g)	Percentage of yield
A	1-3	0.05	1.20
B	4	0.04	1.33
C	5-6	0.06	1.79
D	7-9	0.12	3.92
E	10-12	0.11	3.57
F	13	0.13	4.20
G	14-16	0.52	16.84
H	17-21	0.45	14.47



(a) (b) (c)

Figure 2. TLC profiles of fractions A-H of *Kemuning* leaves extract observes under UV light at wavelength a) 254 nm, b) 365 nm, and c) after spraying with citrate-borate

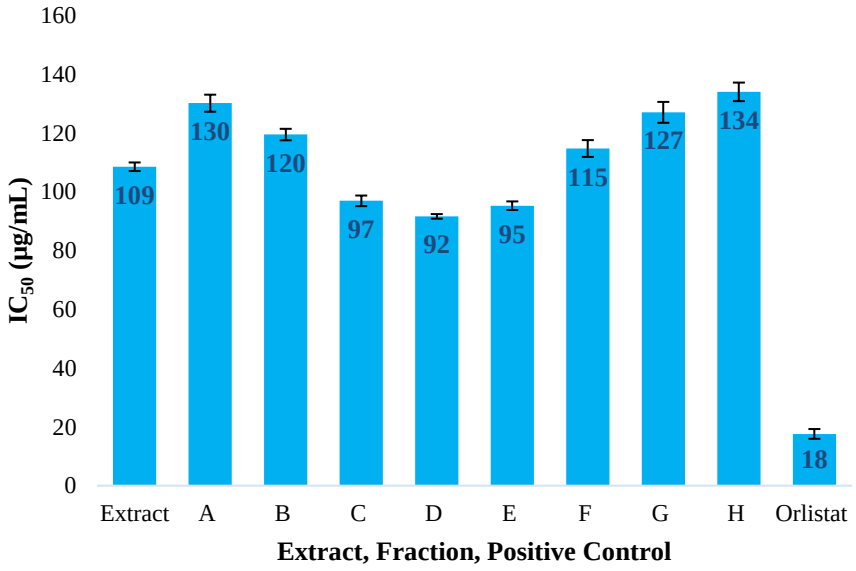


Figure 3. Anti-lipase activity of extracts, fractions, and positive control

Anti-lipase activity

The anti-lipase activity test of *Kemuning* leaves extract and its fraction were shown in Figure 3.

Discussion

Several studies of *Kemuning* leaves extraction show a different yield. The powered *Kemuning* leaves were extracted in 80% ethanol for fifteen days, and after evaporation to dryness, a yield of 3.71% of the crude extract was found (Shaker *et al.,* 2009). Gautam *et al*. (2012) found yield results of 9.5% w/w by extracting *M. paniculata* leaves with 50% ethanol. The yield of *M. paniculate* leaves, which was extracted with 96% ethanol (1:10 w/v) for 24 hours, was 2.87% (Tresiaa *et al.*, 2016). The extraction of *M. paniculate* leaves by maceration using ethanol (96%, 70%, and 40%) yielded 33.45%; 27.60%; and 25.98%. (Wardani *et al.*, 2019). According to the Indonesian Herbal Pharmacopoeia, second edition, *M. paniculata* leaves extracted with 70% ethanol yielded at least 11.1% (Ministry of Health Republic of Indonesia, 2017)

The difference in yield is affected by various factors, including particle size, solvent type, temperature, extraction time, and the solvent to simplicia ratio. Particle size is an important factor to consider making the next extraction stage more efficient. Reducing the particle size will increase its surface area, and the higher the surface area of the particles, the faster the solute transfers from the surface of the solid particle to the solvent. In addition, the large surface area of the particles reduces the intraparticle diffusion paths, leading to more efficient extraction. However, there is a limit to particle size reduction. If it is too fine, it can produce compression of the particle layer and solvent flow, which can reduce the efficiency of the extraction process.

In the extraction process, the type of solvent is the main parameter that affects the efficiency of the process because it determines two important factors, namely the solubility of the target compound and its penetration into the matrix. Therefore, there is no universal solvent for extracting bioactive compounds from natural products because a specific solvent is required for each raw material and target compound.

Temperature affects the solubility and diffusivity of solutes as well as the viscosity and surface tension of liquids. In addition, temperature can provide the necessary energy to disrupt intermolecular interactions between raw material components and make target components available for extraction. Likewise, the extraction time, although extending the process increases the yield for too long time can lead to the degradation of the desired compound. Similarly, increasing the extraction period, although increasing the yield, can lead to the degradation of the desired compound.

The extraction yield tends to increase with the ratio of solvent to simplicia. High ratios can be applied in single-step extraction, whereas lower ratios can be used in multi-step processes. However, the high ratio also causes a high consumption of solvent and energy for solvent removal. Therefore, the ratio must be as low as possible while still ensuring the desired result for the process (Rostagno and Prado, 2013).

The anti-lipase activity test revealed that fractions C, D, and E exhibited stronger anti lipase activity than the extracts and other fractions. These fractions contain semipolar compounds because they are dissolved in a semipolar solvent (a mixture of n-hexane and ethyl acetate). According to the chromatogram profile (Figure 3), the C, D, and E fractions contained flavonoid, as shown by the change in color of the stain to yellow when sprayed with borate citrate. Flavonoids have numerous hydroxyl groups that can form hydrogen bonds with lipase (Li *et al.,* 2023), which exerts an inhibitory effect on pancreatic lipase enzymes through a competitive inhibition mechanism (Tran *et al.*, 2024). The *in-silico* binding model shows that the flavonoid

phenyl ring binds to the aromatic lipase ring (Phe78, Pro181, Tyr115, and Phe216) through a hydrophobic bond. In addition, flavonoids also bind to the catalytic sites of histidine (His264) and serine (Ser153). All flavonoid interactions within the lipase active site explain the inhibitory power of flavonoids, decreasing and reducing fat absorption (Mahboob *et al.*, 2023).

The IC_{50} value represents the minimal concentration of a sample that is required for 50% inhibition in vitro and indicates the potency of inhibiting the lipase. The lower the IC_{50}, the stronger the inhibitory strength of a sample. Figure 2 depicts the IC_{50} for each sample.

According to Akhtar et al. (2019), if the IC_{50} of the sample is ≤ 10 times the IC_{50} of the positive control, it can be categorized as very strong; strong, between 10 and 25 times; moderate, between 25 and 50 times; and > 50 times, it can be categorized as weak. Based on these categories, *Kemuning* leaves extract and its fractions have very strong activity to inhibit lipase.

Conclusion

Based on the findings of the research, it is possible to conclude that *Kemuning* leaves extract and its fractions can significantly decrease lipase activity. The fractions C, D, and E were more potent than the extracts.

Conflict of Interest

The authors hereby declare that regarding the publication of this paper there is no conflict of interests.

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Authors Contribution

The first author developed the research design, collected data, and prepared a draft manuscript. The second author assisted with data collection. The third and fourth authors analyzed the data. The fifth author revised and finalized the manuscript for publication.

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