

# 2024\_Antioxidant Activity and Pancreatic Lipase Inhibition

*by Ririn Suharsanti*

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**Antioxidant Activity and Pancreatic Lipase Inhibition of *Curcuma aeruginosa* Roxb Rhizome Fractions**Suharsanti<sup>1</sup>, Subagus Wahyuono<sup>2</sup>, Nunung Yuniarti<sup>3</sup>, Puji Astuti<sup>4\*</sup>

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<sup>1</sup>Doctoral Program of Pharmaceutical Science, Faculty of Pharmacy, Universitas Gadjah Mada, Jl Sekip Utara, Mlati, Sleman, Yogyakarta, Indonesia, 55281. <sup>2</sup>STIFAR Yayasan Pharmasi, Letjend Sarwo Edie Wibowo KM 1, Semarang, Indonesia, 50192. <sup>3,4</sup>Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Jl Sekip Utara, Mlati, Sleman, Yogyakarta, Indonesia. <sup>3</sup>Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Jl Sekip Utara, Mlati, Sleman, Yogyakarta, Indonesia, 55281

e-mail:

<sup>1</sup>ririnsuharsanti@stifar.ac.id<sup>2</sup>subagusw\_fa@ugm.ac.id<sup>3</sup>nunungyuniartiwidodo@gmail.com<sup>4</sup>puji\_astuti@ugm.ac.id

\*Corresponding author

**Abstract.** *Antiobesity with a lipase inhibitor mechanism will block the hydrolysis of triglycerides into fatty acids and glycerol, while antioxidant compounds are very useful in conditions of obesity to prevent excess damage from degenerative diseases. Curcuma aeruginosa Roxb extract has been proven to inhibit pancreatic lipase, so further effects will be seen at the fraction level. This research aims to investigate the phytochemical components, antioxidant activity and pancreatic lipase inhibition of Curcuma aeruginosa Roxb fractions. Ethanol extract of the Curcuma aeruginosa Roxb rhizome was separated using the HPLC-liquid chromatography with 3 different solvents (n-hexane, ethyl acetate, ethanol) to give n-hexane (HF), ethyl acetate (EAF), and ethanol (EF), and the insoluble (IF) fractions. Each fraction detected phenolics, flavonoids, alkaloids, tannins, saponins, triterpenoids/steroids. EF has the highest total flavonoid and phenolic content. Antioxidant activity of all fractions were measured using DPPH reduction, ABTS, and FRAP methods. The best antioxidant activity of all fractions using the DPPH method was EF with IC<sub>50</sub> 21.93 ± 3.39 µg/mL, ABTS method was HF with IC<sub>50</sub> 24.56 ± 1.03 µg/mL and FRAP method was IF with IC<sub>50</sub> 20.79 ± 1.03 µg/mL. The total number of phenolics and flavonoids in EF strongly supports the antioxidant activity of the DPPH method. The highest inhibition of pancreatic lipase was found in EAF at 35.16 ± 0.24 % (100 µg/ml). There was significant difference between EAF and xenical (orlistat) (p < 0.05).*

**Keywords:** antioxidant, curcuma aeruginosa, fraction, pancreatic lipase inhibition

**Citation**

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## INTRODUCTION

According to the World Health Organization's World Obesity Atlas 2022, Indonesia is ranked 131st out of 183 countries for readiness to face obesity, reflecting a poor "global preparedness ranking" (Lobstein et al., 2023). Data from the 2018 Basic Health Research (Kemkes RI, 2018) indicates that obesity in Indonesia increased from 14.8% in 2013 to 21.8% in 2018. Obesity is a priority health indicator in the 2020-2024 national medium-term development plan, aiming to maintain the prevalence at 21.8% by the end of 2024. Obesity has become a risk factor for various diseases, including cardiovascular conditions, endocrine gland disorders, gastrointestinal disorders, reproductive disorders, erectile dysfunction, and dermatological problems.

Obesity treatment includes inhibiting pancreatic lipase and suppressing appetite in the central nervous system (CNS) (Brown et al., 2009). Pancreatic lipase (PL) inhibitors play an essential role in human fat metabolism (Liu et al., 2020). Oxidative stress resulting from high levels of triglycerides, associated with obesity, interfere with the metabolism as well as oxygen exchange function in adipocytes mitochondria, leading to the generation of superoxide (Fernández-Sánchez et al., 2011). Obesity triggers inflammatory processes, excessive lipogenesis, inhibition of lipolysis, and increased adipocyte apoptosis, leading to the release of Reactive Oxygen Species (ROS) and further oxidative stress. This stress can cause cell and tissue damage, potentially resulting in degenerative diseases.

Compounds in *Curcuma aeruginosa* Roxb. rhizomes exhibit antioxidant and anti-obesity activities in vitro, offering potential benefit. The antioxidant properties help prevent more severe diseases associated with obesity. Orlistat, a medication for obesity,

works by preventing the body from absorbing fat in the digestive tract (Zhang et al., 2014). With lipase inhibitors, the hydrolysis of triglycerides into fatty acids and glycerol is prevented (Liu et al., 2020). While anti-obesity drugs considered safe, its use is limited due to side effects. Anti-obesity drugs targeting the central nervous system often lead to side effects such as constipation, diarrhea, headaches, fatigue, and even cancer risk (Mauer et al., 2021). Orlistat's main drawback includes digestive side effects, such as abdominal discomfort, loose stools, soft stools, oily rectal spotting, flatulence and flatulence with discharge, stool urgency, fatty or oily stools, increased defecation, and fecal incontinence (Aberg et al., 2009; Heck et al., 2000).

*Curcuma aeruginosa* has been used traditionally to regulate body weight after childbirth or menstruation (Mardisiswojo & Rajakmangunsudarso, 1985), and some of its ingredients may have a weight-reducing effect (Permadi, 2008). Previous research shows that the antilipase activity of *Curcuma aeruginosa* methanol extract was  $29.6 \pm 0.2\%$  demonstrating the best activity among other rhizomes tested (Alias et al., 2017). However, no research has explored this activity at the fraction level. Furthermore, no reports identify compounds in *Curcuma aeruginosa* that may play a role in obesity treatment. Its antioxidant properties have only been investigated at the fraction level (Sugita et al., 2018). This study aimed to identify the fraction's phytochemical compounds and determine its antioxidant activity ( $IC_{50}$ ) and pancreatic lipase inhibitory properties (%).

## MATERIALS AND METHODS

### Materials

*Curcuma aeruginosa* Roxb rhizomes were obtained from the Center for Research

and Development of Traditional Medicinal Plants and Medicines Tawangmangu, Central Java, Indonesia, with collection number CA-01-03-20 and harvested in February (2000). Ethanol, methanol, n-hexane, ethyl acetate was used as solvents (Smart Lab, Indonesia). Other materials included folin-ciocalteu, sodium carbonate, aluminum chloride, sodium acetate, gallic acid, and standard quercetin as standard, DPPH, TPTZ (Sigma-Aldrich; St. Louis, MO, USA), Porcin pancreatic lipase (PPL) (Type II) (EC 3.1.1.3) (Sigma-Aldrich; St. Louis, MO, USA), P-nitrophenyl butyrate (pNPB) (Sigma-Aldrich; St. Louis, MO, USA), and potassium phosphate buffer pH 7.2 were also purchased from Sigma, Aldrich through PT. Kairos Indonesia suppliers. Xenical containing orlistat was obtained from Kimia Farma Drugstore, Indonesia.

## Methods

### Extraction and Fractination

Dried *Curcuma aeruginosa* rhizome was powdered, and 1 kg of powdered material was macerated with 70% ethanol in a ratio of 1:5 and left overnight for 3 days. Upon filtration, the filtrates were combined and evaporated to dryness using a rotary vacuum evaporator at 60°C and 100 rpm, yielding an ethanol extract (EE). The ethanol extract (EE) was then separated by solid (SiO<sub>2</sub>)-liquid chromatography, employing solvents ranging from non-polar (n-hexane), semi-polar (ethyl acetate), to polar (ethanol) to obtain n-hexane (FH), ethyl acetate (EAF), ethanol (EF), and insoluble fractions (IF). Thin layer chromatography (SiO<sub>2</sub> PF) was used to identify compounds present in the extracts and fractions, with a mobile phase of chloroform: methanol: glacial acetic acid (94:5:1) and visualized under UV light and cerium sulfate.

## Phytochemical Analysis

### Qualitative Phytochemical Screening

#### a) Phenolic

One mL of EE, HF, EAF, EF, and IF was taken, and 5 drops of 5% FeCl<sub>3</sub> solution were added. The appearance of a blue-black color indicates the presence of phenolic compounds in the extract (Jemal et al., 2022).

#### b) Flavonoids

One gram of EE, HF, EAF, EF, and IF was placed in a water bath. Magnesium powder was then added, followed by 1 mL of concentrated HCl and amyl alcohol. The mixture was shaken vigorously, and allowed to separate. A red, yellow, or orange color in the amyl alcohol layer indicates the presence of flavonoids (Faramayuda et al., 2021).

#### c) Alkaloids

One gram of EE, HF, EAF, EF, and IF was mixed with 9 mL of distilled water and 1 mL of 2N HCl, then heated in a water bath for two minutes and filtered. The filtrate was divided into three test tubes. In the first tube, two drops of Mayer's reagent were added, forming a white or yellow precipitate. In the second tube, two drops of the Boucard reagent were added, producing a brick-red precipitate, confirming the presence of alkaloids. For the third tube, two drops of Dragendroff reagent were added, producing a brick red precipitate, confirming the presence of alkaloids (Dilshad & Batool, 2022; Jemal et al., 2022).

#### d) Tannins

One gram of EE, HF, EAF, EF, and IF was mixed with 10 mL of hot distilled water, followed by the addition of 1 mL of 10% NaCl. The mixture was filtered, and the filtrate was divided into two test tubes. The first tube was added with a few drops of FeCl<sub>3</sub> 5% to ensure positive indication of phenolic compounds. The second tube was dripped with three drops of 0.5% gelatin solution. A white precipitate

indicates the presence of tannins (Bonetti et al., 2020).

e) Saponins

One gram of EE, HF, EAF, EF, and IF was dissolved in warm water, and a little amount of each sample was added to a test tube with 10 mL of distilled water. The test tube was tightly sealed and shaken vigorously to produce foam. The foam was drizzled with 2 N HCl up to a height of 1 cm. If the foam height remains unchanged, this suggests the presence of saponins (Dilshad & Batool, 2022; Jemal et al., 2022).

f) Triterpenoids/Steroids

One gram of EE, HF, EAF, EF, and IF was pre-dissolved in n-hexane. A portion was placed in a test tube, and 1 mL each of glacial acetic acid and concentrated H<sub>2</sub>SO<sub>4</sub> (Liebermann-Burchard reagent) was added. The formation of a brown ring at the interface indicates the presence of triterpenoids, while a blue or green ring suggests the presence of steroids (Dilshad & Batool, 2022).

#### Quantitative Phytochemical Screening

##### a) Determination of Total Phenolic Content (TPC)

The total phenolic content was determined using a colorimetric method (Nurcholis et al., 2016; Rahardhian et al., 2019). EE, FH, EAF, EF and IF were dissolved in ethanol p.a. up to a total volume of 10 mL. To 0.5 mL of each sample solution, 0.4 mL of Folin-Ciocalteu reagent was added, and the mixture was incubated for 4-8 minutes. Then, 4.0 mL of 7% sodium carbonate solution and 10 mL of distilled water were added. After 2 hours of incubation at room temperature, the absorbance was measured at 750 nm using a UV-Vis spectrophotometer (Shimadzu UV-1280, Japan). A calibration curve was prepared with standard solutions of gallic acid at concentrations of 40, 60, 80, 100, and

120 mg/mL. The total phenolic content was expressed as mg of gallic acid equivalent per g of sample.

##### b) Determination of Total Flavonoid Content (TFC)

The total flavonoid content was determined by the colorimetric method (Nurcholis et al., 2016; Rahardhian et al., 2019). Each sample (10 mg of EE, FH, EAF, EF, and IF) was dissolved in ethanol p.a. up to a volume of 10 mL. Then, 0.5 mL of each sample solution was mixed with 2.5 mL of distilled water, 0.1 mL of 10% AlCl<sub>3</sub>, 0.1 mL of 1 M sodium acetate, and 1.5 mL of methanol. After 30 minutes of incubation, absorbance was measured at 415 nm using a UV-Vis spectrophotometer (Shimadzu UV-1280, Japan). The concentrations of 40, 60, 80, 100, and 120 g/mL of quercetin were used as the standard for the calibration curves, and the findings were reported as quercetin equivalents in mg of quercetin per g of sample.

##### DPPH Radical Scavenging Assay

A total of 10 mg of EE, FH, EAF, EF, and IF was diluted with methanol in a 10 mL flask, and a concentration series was prepared from each solution. For each concentration in the series, 0.2 mL of the sample solution was placed in a test tube, and 4.0 mL of 0.1 mM DPPH solution was added. The mixture was vortexed for one minute to ensure homogeneity and then left to stand for 30 minutes. Absorbance was measured at a wavelength of 517 nm. The same procedures were performed with a quercetin standard series, with concentrations ranging from 10 to 50 mg/mL, for comparison (Nurcholis et al., 2016; Rahardhian et al., 2019). The IC<sub>50</sub> value, representing the concentration needed to scavenge 50% of the DPPH free radicals, was determined. The percentage

of antioxidant activity was calculated by comparing the absorbance of EE, FH, EAF, EF, and IF with the absorbance of the DPPH control using the following formula:

$$\% \text{ aktivitas antioksidan} = \frac{\text{Abs. kontrol} - \text{Abs. sampel}}{\text{Abs. kontrol}} \times 100\%$$

#### Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP solution was prepared by mixing 10 parts of 0.1 M acetate buffer (pH 3.6), 1 part of a 40 mM HCl solution containing TPTZ (2,4,6-tripyridyl-s-triazine), and 1 part of 12 mM FeCl<sub>3</sub>·6H<sub>2</sub>O solution. Each of the EE, FH, EAF, EF, and IF samples (10 mg) were diluted with methanol in a 10 mL flask, and a concentration series was created from each. For each sample series, 0.2 mL of the FRAP solution and 0.4 mL of the sample solution were added to a test tube. The mixture was vortexed for 1 minute to homogenize it, and then it was incubated in a dark room for 10 minutes. Absorbance was measured at a wavelength of 595.4 nm. A quercetin standard solution, with concentrations ranging from 5 to 10 mg/mL, was also measured. The ferric reducing antioxidant power (FRAP) was determined by comparing the sample's absorbance with that of a 12 mM FeSO<sub>4</sub> standard, which served as the 100% FRAP reference (Benzie & Strain, 1996; Setiawan et al., 2021). The percentage of antioxidant activity was calculated using the following formula:

$$\% \text{ FRAP} = \frac{\text{Nilai FRAP senyawa}}{\text{Nilai FRAP FeSO}_4 \text{ 100\%}} \times 100\%$$

#### ABTS Radical Scavenging Assay

A total of 10 mg of EE, FH, EAF, EF, and IF was diluted in methanol in a 10 mL flask. A concentration series was then prepared for

each solution. To assess antioxidant activity, 4.0 mL of ABTS solution was added to a test tube, followed by 0.2 mL of each concentration of the extract solutions (EE, FH, EAF, EF, and IF). The mixture was vortexed for 1 minute to ensure homogeneity and then allowed to stand in the dark for 6 minutes, as specified by the protocol. Absorbance was measured at a wavelength of 736 nm. The same procedure was applied to a quercetin standard series with concentrations ranging from 5 to 10 mg/mL. Antioxidant activity was determined by comparing the absorbance values of EE, FH, EAF, EF, and IF to the ABTS control solution (Mukherjee et al., 2011; Rajurkar & Hande, 2011) using the following formula:

$$\% \text{ aktivitas antioksidan} = \frac{\text{Abs. kontrol} - \text{Abs. sampel}}{\text{Abs. kontrol}} \times 100\%$$

#### Pancreatic Lipase Inhibition Assay

An ELISA reader was used to measure the hydrolysis of pNBP to p-nitrophenol at a wavelength of 405 nm in UV-transparent 96-well plates, providing data on lipase activity (BIO-TEK, Synergy HT, USA). Each extract (EE, FH, EAF, EF, and IF) was prepared at a concentration of 100 mg/mL. To create the porcine pancreatic lipase (PPL) solution, an enzyme solution at 1 mg/mL concentration was prepared in 50 mM phosphate buffer at pH 7, then centrifuged for 5 minutes to remove insoluble components. For the reaction, the enzyme concentration was adjusted to 0.1 mg/mL. ELISA plate reader was used to measure the hydrolysis of pNBP to p-nitrophenol at a wavelength of 405 nm in UV-transparent 96-well plates, providing data on lipase activity (BIO-TEK, Synergy HT, USA). Each extract (EE, FH, EAF, EF, and IF) was prepared at a concentration of

100 mg/mL. To create the porcine pancreatic lipase (PPL) solution, an enzyme solution at 1 mg/ml concentration was prepared in 50 mM phosphate buffer at pH 7, then centrifuged for 5 minutes to remove insoluble components. For the reaction, the enzyme concentration was adjusted to 0.1 mg/mL.

The pNPB substrate was initially dissolved in 1% dimethyl sulfoxide (DMSO) and then diluted in 50 mM phosphate buffer (pH 7.2) to a concentration of 2.5 mM. Each sample received 50 µL of the prepared sample solution and was incubated at 37°C for 10 minutes. After incubation, additional substrate and buffer were introduced, and the solution was incubated once more at 37°C for another 10 minutes. Orlistat was used as a positive control, containing the same enzyme and substrate as the test samples, while DMSO served as a negative control, both with and without inhibitors. Measurements of both the positive and negative controls, along with blank measurements, were conducted at 415 nm without enzymes and substrates added (Alias et al., 2017). One activity unit was defined as the rate of reaction producing one mole of nitrophenyl butyrate at 37°C. The lipase inhibition percentage was calculated by determining the reduction in lipase activity, using the following formula:

$$\text{Inhibition of lipase (1\%)} = 100 - \left[ \frac{(B - b)}{(A - a)} \times 100 \right]$$

Where A represents the activity in the absence of inhibitor, a is the negative control without inhibitor, B is the activity with inhibitor, and b is the negative control with inhibitor.

#### Statistical Analysis

Three sets of data were collected for each experiment, and the results were

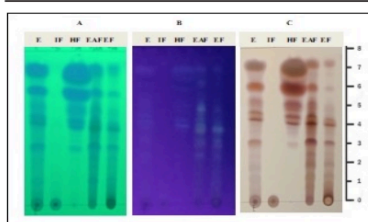
presented as mean and standard deviation. Three independent data sets were collected for each experiment, and the results are presented as mean ± standard deviation. A one-way analysis of variance (ANOVA) was conducted to assess differences between the groups using SPSS statistical software (SPSS 23.0). A p-value of <0.05 was considered the threshold for statistical significance.

## RESULTS AND DISCUSSION

### Extraction and Fractionation

The extraction of *Curcuma aeruginosa* rhizome using the maceration method yielded 21.8%. Previous research reported yields from *Curcuma aeruginosa* Roxb harvested from 20 locations in Indonesia ranging from 7.92% to 19.71% (Nurcholis et al., 2016). Fractionation was performed sequentially with n-hexane, ethyl acetate, and ethanol, resulting in yields of 14.6% for HF, 17.5% for EAF, 12.2% for EF, and 49.4% for IF. Based on these yields, the insoluble fraction (IF) appears to contain many very polar compounds.

Extracts and fractions were analyzed using thin-layer chromatography, with simultaneous spotting on the plate and elution in a chloroform:methanol:glacial acetic acid (94:5:1) solvent system. The chromatographic pattern was observed under UV light at 254 nm and 366 nm and visualized with cerium sulfate. The n-hexane fraction (HF) showed non-polar compounds with  $R_f > 0.50$ , while ethyl acetate and ethanol fractions had more polar compounds, showing spots with  $R_f \leq 0.50$ . The IF fraction contained highly polar compounds that remained at the baseline ( $R_f = 0$ ). Overlapping spots were noted in HF, EAF, and EF. The chromatographic patterns of the extracts and fractions are shown in Figure 1.



**Figure 1.** Thin layer chromatography results of *Curcuma aeruginosa* Roxb extracts and fractions. The spots are visualized using UV 254 nm (A), UV 366 nm (B), and cerium sulfate (C). E=extract, IF=insoluble fraction, HF=n-hexane fraction, EAF=ethyl acetate fraction, EF=ethanol fraction

### Qualitative Analysis of Phytochemicals

During fractionation, compounds were separated based on polarity, resulting in distinct fractions: EE, HF, EAF, EF, and IF from *Curcuma aeruginosa*. Qualitative phytochemical screening indicated the presence of phenolics, flavonoids, alkaloids,

tannins, saponins, and triterpenoids/steroids in all fractions (Table 1). The consistent presence of these phytochemicals across fractions suggests some overlap in compound composition, likely due to the simplicity of the separation method. Advanced techniques, such as flash chromatography and preparative HPLC, could further enhance compound separation in future studies.

Previous studies have shown that compounds such as polyphenols, flavonoids, catechins, proanthocyanidins, saponins, and triterpenes influence the inhibition of pancreatic lipase (De La Garza et al., 2011). Additionally, earlier research on the primary, secondary, and tertiary rhizomes of *Curcuma aeruginosa* reported that saponins were detectable in the 70% ethanol and aqueous extracts, but not in the 96% ethanol extracts. Alkaloids, flavonoids, and steroids were consistently present in fractions obtained from various parts of the *Curcuma aeruginosa* rhizome (Nurcholis et al., 2015).

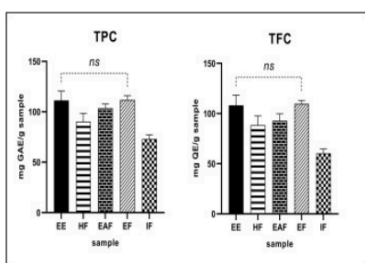
**Table 1.** Qualitative Phytochemical Screening of *Curcuma aeruginosa* Extract and Fractions

Phytochemical	Reagent	EE	HF	EAF	EF	IF
Phenolics	Ferric chloride test	+	+	+	+	+
Flavonoids	Amyl alcohol test	+	+	+	+	+
	Mayer test	+	+	+	+	+
Alkaloids	Deagendorf test	+	+	+	+	+
	Bouchardat test	+	+	+	+	+
Tannins	Gellatins test	+	+	+	+	+
Saponins	Foam test	+	+	+	+	+
Triterpenoids/steroids	Liebermann test	+	+	+	+	+

Note: EE=ethanolic extract, HF=hexan fraction, EAF=ethylacetate fraction, EF=ethanol fraction, IF=insoluble fraction

### Quantitative Analysis of Phytochemicals

Quantitative analysis of phytochemicals focused on two classes of compounds: phenolics and flavonoids, by measuring their total content. Both classes are essential in various activities, including antioxidant processes. The total phenolic content (TPC) of the EE extract and HF, EAF, EF, and IF fractions varied, with EF showing the highest TPC, followed by EE, EAF, HF, and IF, which had the lowest TPC (Figure 2). There was no significant difference in TPC between EF and EE. The high TPC values in EE, EF, and EAF likely result from similar compounds, as seen in the TLC profile (Figure 1). The residual fraction (IF) showed the lowest TPC because phenolic compounds had already been extracted into previous fractions.



**Figure 2.** Total phenolics contents/TPCs (mg GAE/g sample) and total flavonoids contents/TFCs (mg QE/g sample) of ethanolic extract (EE), hexan fraction (HF), ethyl acetate fraction (EAF), ethanol fraction (EF), insoluble fraction (IF). Values are mean  $\pm$  SD (n = 3), ns= not significant ( $\alpha > 0.05$ )

The TFC results mirrored the TPC results. The TFC results have the same pattern as the TPC results, with the highest value in EF, followed by EE, EAF, HF, and IF, which showed the lowest TFC, consistent with the TPC pattern (Figure 2). There was no significant

difference ( $p > 0.05$ ) in TFC between EF and EE based on ANOVA analysis, while other groups showed significant differences ( $p < 0.05$ ). The TPC and TFC results in this study are higher than those reported in previous studies on *Curcuma aeruginosa* harvested in Indonesia. Previous studies found variability in TPC and TFC, with values ranging from 29.08–46.92 mg GAE/g and 21.31–33.81 mg QE/g, respectively (Nurcholis et al., 2019). Thus, this accession holds potential for commercial use and traditional medicine preparations. The phenolic and flavonoid compounds identified are polar, with the ethanol fraction (EF) containing the highest amounts (Kodjio et al., 2016; Nisar et al., 2015).

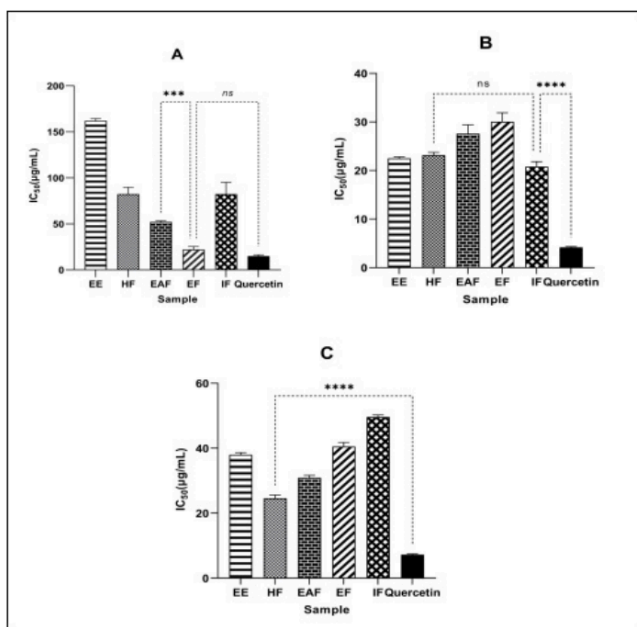
### DPPH Radical Scavenging Assay

DPPH, an unstable nitrogen free radical, can bind hydrogen ions, allowing it to test antioxidant activity. The presence of hydrogen ions in the solution results in a color change from dark purple to yellow in DPPH methanol solution (Rahardhian & Suharsanti, 2019). Quercetin was used as the standard in this study. Results showed that the extract had the lowest antioxidant activity. Using the DPPH method, EF exhibited the best activity with an IC<sub>50</sub> of  $21.93 \pm 3.39$   $\mu$ g/mL, while quercetin showed an IC<sub>50</sub> of  $14.90 \pm 1.44$   $\mu$ g/mL. There was no significant difference ( $p > 0.05$ ) between EF and quercetin, while a significant difference ( $p < 0.001$ ) was observed between EAF and EF after ANOVA analysis (Figure 3). The total phenolic and flavonoid content in EF supported antioxidant activity. The IF fraction, having the lowest TPC and TFC, also showed the lowest antioxidant activity in the DPPH assay.

The antioxidant activity of flavonoids is mainly due to phenolic hydroxyl groups in their molecular structure. When these

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 compounds react with free radicals, they form new radicals stabilized by the resonance effect of aromatic nuclei (Rahardhian & Suharsanti, 2019). Compared with other studies on the antioxidant activity of *Curcuma aeruginosa* rhizomes across 20 accessions in Indonesia, this study found lower activity levels, as previous samples showed IC<sub>50</sub> values of 0.34–19.59% (Nurcholis et al., 2016). Differences in

harvest location may account for variations in compound content and activity. Prior research indicated that the DPPH assay for antioxidant activity in *Curcuma aeruginosa* rhizome extracts yielded superior results compared to other methods, including ABTS, superoxide anion scavenging assay, phosphomolybdenum assay, and hydroxyl radical scavenging assay (Zohmachhuana et al., 2022).



**Figure 3.** (A) DPPH radical scavenging activity, (B) FRAP (Ferric Reducing Antioxidant Power) activity, and (C) ABTS scavenging activity of ethanolic extract (EE), n-hexane fraction (HF), ethyl acetate fraction (EAF), ethanol fraction (EF), insoluble fraction (IF), and quercetin in IC<sub>50</sub> (µg/mL). Values are mean ± SD (n = 3). \*\*\* and \*\*\*\* are significant ( $\alpha < 0.05$ ), ns = not significant ( $\alpha > 0.05$ )

### Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing/antioxidant power (FRAP) assay is a standard method for assessing antioxidant activity in foods (Sudha & Srinivasan, 2014). Reducing capabilities are generally linked to the presence of compounds that act by breaking free radical chains through the donation of hydrogen atoms. The FRAP method measures the ability of antioxidants to reduce ferri-tripyridyl-triazine to the complex ferri-tripyridyl-triazine (Fe(II) TPTZ), enabling the quantification of antioxidant activity (Benzie & Strain, 1996). In this study, the IF fraction exhibited the highest reduction/antioxidant activity, with an  $IC_{50}$  of  $20.79 \pm 1.03 \mu\text{g/mL}$ , while EF had the lowest reduction/antioxidant activity, with an  $IC_{50}$  of  $29.17 \pm 1.57 \mu\text{g/mL}$ . The  $IC_{50}$  of IF using the FRAP method did not surpass the  $IC_{50}$  of EF in the DPPH test. IF, which showed the strongest reducing ability, had a statistically significant difference ( $p < 0.0001$ ) when compared to quercetin, the standard. HF demonstrated the next greatest reduction capability after IF, with an  $IC_{50}$  of  $23.12 \pm 0.62 \mu\text{g/mL}$ , followed by EAF at  $27.59 \pm 1.82 \mu\text{g/mL}$ . Statistical analysis revealed no significant difference ( $p > 0.05$ ) between IF and HF (Figure 3). The FRAP assay results differ from those of the DPPH antioxidant test. The FRAP and DPPH assays yielded opposite patterns, with EF having the highest activity against DPPH radicals but low reducing power in the FRAP test, while IF showed strong reducing power in the FRAP test and low ability to scavenge DPPH free radicals. This suggests that the high phenolic and flavonoid content in EF (Figure 2) contributes to its DPPH scavenging effect. These findings contrast with research by Simoh et al. (2018), which reported that phenolics from *Curcuma aeruginosa* rhizome extract exhibited higher reducing power

than radical scavenging ability. Phenolic compounds generally function as antioxidants, with activity that depends on their chemical characteristics and mechanisms of action, such as metal chelation, hydrogen donation, or oxygen quenching (Galato et al., 2001).

### ABTS Radical Scavenging Assay

The ABTS radical cation (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) test is widely used to assess antioxidant capacity (Ilyasov et al., 2020). Antioxidant activity is determined by measuring cation absorption (Re et al., 1999). In the ABTS assay, results varied from those of the DPPH test. While EF showed the highest activity in the DPPH test, HF had the strongest activity in the ABTS test, with an  $IC_{50}$  of  $24.56 \pm 1.03 \mu\text{g/mL}$ , followed by EAF at  $30.91 \pm 0.74 \mu\text{g/mL}$ , EF at  $40.51 \pm 1.26 \mu\text{g/mL}$ , and IF at  $49.62 \pm 0.6 \mu\text{g/mL}$ , which had the lowest activity. The antioxidant activity of HF did not surpass that of the quercetin standard, and statistical analysis showed a significant difference ( $p < 0.0001$ ) between HF and quercetin based on ANOVA (Figure 3).

The ABTS results are not markedly superior to those of the DPPH test. Some studies suggest that the ABTS assay may be more suitable than DPPH for evaluating antioxidant capacity in various foods (Floegel et al., 2011). A study by Zohmachhuana et al. (2022) indicated that rhizome samples had higher antioxidant activity than other plant parts in DPPH and ABTS tests. Other research has noted different trends in antioxidant activity measurements between DPPH and ABTS, with additional hydroxyl (OH) groups enhancing DPPH activity but not affecting ABTS as strongly. A sugar residue had a slightly negative effect in the ABTS test but no significant impact in the DPPH test (Platzer et al., 2021). Similarly, EF, being a polar

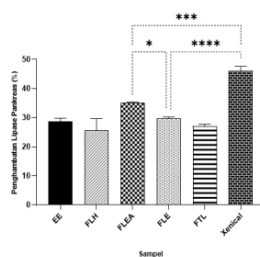
fraction with many OH groups, exhibited high antioxidant activity in the DPPH assay. In this study, ABTS radicals react more quickly than DPPH radicals due to higher reactivity and sensitivity, leading to lower  $IC_{50}$  values in the ABTS method (Theafelicia & Wulan, 2023).

#### Pancreatic Lipase Inhibition Assay

Various methods are available to test lipase activity, using either natural or synthetic triglycerides as substrates. These methods include spectrophotometric, turbidimetric, titrimetric, chromogenic, and immunochemical techniques (Alias et al., 2017). In this study, the pancreatic lipase inhibitory activities of the EE extract and HF fraction were evaluated, with EAF, EF, and IF obtained through successive spectroscopic fractionation using pNPB as a substrate. Tests were performed in a 96-well plate and measured using a microplate reader. The hydrolysis of pNPB (p-nitrophenyl butyrate) releases p-nitrophenolate, which is detectable spectrophotometrically at 415 nm at various time intervals (Pliego et al., 2015). Among the fractions, EAF demonstrated the highest pancreatic lipase inhibition activity at  $35.15 \pm 0.24\%$ , followed by EF at  $29.66 \pm 0.57\%$ , IF at  $27.03 \pm 0.73\%$ , and HF at  $25.51 \pm 4.14\%$ . However, EAF's inhibition was lower than that of the positive control, orlistat, which had an inhibition rate of  $46.07 \pm 1.54\%$  (Figure 4).

A prior study found that an 80% methanol extract from *Curcuma aeruginosa* rhizome had a similar lipase inhibition rate, around  $29.6 \pm 0.20\%$  (Alias et al., 2017). No other studies have examined the pancreatic lipase inhibition activity of the *Curcuma aeruginosa* rhizome fraction. A significant difference ( $p < 0.001$ ) was found between EAF and orlistat (xenical) and between EAF and EF ( $p < 0.05$ ) (Figure 3). As a synthetic drug, orlistat is specifically optimized to inhibit

pancreatic lipase effectively and selectively by binding directly to the enzyme's active site, causing strong and irreversible inhibition. In contrast, plant extracts may contain reversible competitive inhibitors that do not have the same potency as orlistat.



**Figure 4.** Pancreatic lipase Inhibitory effect (%) of ethanolic extract (EE), n-hexane fraction (HF), ethyl acetate fraction (EAF), ethanol fraction (EF), insoluble fraction (IF) and xenical as positive control. All groups were significantly different from the control ( $\alpha < 0.05$ ).

EAF and EF showed strong pancreatic lipase inhibition activity compared to other fractions. Based on DPPH antioxidant results and phenolic and flavonoid levels, EAF and EF also contained higher amounts of these compounds than other fractions. The TLC chromatogram in Figure 1 shows similar chemical profiles for EAF and EF, with spots at R<sub>f</sub> 87, 75, 63, 53, 50, 38, 33, 25, 18, and 13, indicating that these fractions share similar constituents. EAF and EF exhibit both antioxidant and anti-lipase activities. The incorporation of such natural antioxidants into the diet, especially from herbal treatments with pancreatic lipase inhibitory effects, may offer a practical way to increase antioxidant intake (Sosnowska et al., 2016). Phenolic

compounds, widely known for their free-radical scavenging properties, can also play a role in weight management by inhibiting pancreatic lipase.

The correlation between antioxidant capacity and pancreatic lipase inhibition suggests that the phenolic compounds in the EF and EAF fractions also contribute to pancreatic lipase inhibition. This enzyme plays a key crucial in fat digestion, so antioxidant capacity and pancreatic lipase inhibition may be interconnected, highlighting these fractions' potential to regulate fat absorption through enzyme inhibition. This is consistent with previous research, which reported a relationship between pancreatic lipase inhibition and the polyphenol content, particularly proanthocyanidins. Higher total phenolic, anthocyanin, and proanthocyanidin levels correlate with greater antioxidant and lipase inhibition activities (Sosnowska et al., 2016). Further, studies have shown that teas with higher phenolic content, such as green tea, exhibit greater antioxidant and lipase inhibition activities (Gulua et al., 2018). Polyphenols are known to inhibit enzymes involved in fat metabolism, including pancreatic lipase, lipoprotein lipase, and glycerol-3-phosphate dehydrogenase (De La Garza et al., 2011).

Antioxidant compounds in *Curcuma aeruginosa* rhizomes, with anti-obesity activity via pancreatic lipase inhibition in vitro, are beneficial. Antioxidants are essential for preventing more severe complications related to obesity. EF and EAF hold promise for development as standardized anti-obesity herbal treatments, though further testing, particularly in vivo, is essential. Such studies could aid in identifying and characterizing chemical lipase inhibitors and developing medicinal formulations for managing obesity to support the use of EF and EAF as

standardized herbal medicines (Calabrone et al., 2015). Several natural compounds with pancreatic lipase inhibitory properties, such as flavonoids, also have strong antioxidant effects. On a molecular level, flavonoids and polyphenols may bind to the lipase active site, modifying its structure or interacting with active site residues. This interaction prevents fat breakdown and also neutralizes free radicals that could otherwise damage cells.

#### CONCLUSION

The EAF and EF fractions demonstrated higher antioxidant capacities and phenolic and flavonoid contents than the others. There were distinct differences in total phenolic content and antioxidant capacity across the four fractions and their extracts. EAF showed the most potent inhibition of pancreatic lipase activity, suggesting that *Curcuma aeruginosa* could be a valuable natural source for obesity treatment. EAF and EF fractions demonstrated higher antioxidant capacities and higher phenolic and flavonoid contents than the other fractions. There were distinct differences in total phenolic content and antioxidant capacity across the four fractions and their extracts. EAF showed the strongest inhibition of pancreatic lipase activity, suggesting that *Curcuma aeruginosa* could be a valuable natural source for obesity treatment.

#### AUTHOR CONTRIBUTION

W.S designed the research and supervised the fractionation and analysis process, A.P, and Y.N supervised the manuscript, and S.R collected and analyzed the data and wrote the manuscript.

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#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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