Antioxidant Activity and Pancreatic Lipase Inhibition of Curcuma aeruginosa Roxb Rhizome Fractions

by Ririn Suharsanti

Submission date: 27-Feb-2025 09:47PM (UTC+0700)
Submission ID: 2427977157
File name: Biodjati_33900-114487-1-PB.pdf (683.88K)
Word count: 7592
Character count: 41431

Jurnal Biodjati 9 (2): 228-243,November 2024 e-ISSN : 2541-4208
p-ISSN : 2548-1606

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

Antioxidant Activity and Pancreatic Lipase Inhibition of *Curcuma aeruginosa* Roxb Rhizome Fractions

Suharsanti[1], Subagus Wahyuono[2], Nunung Yuniarti[3], Puji Astuti[4*]

Received: 28 February 2024
Revise from: 17 March 2024
Accepted: 02 November 2024

DOI: 10.15575/biodjati.v9i2.33900

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

e-mail:
[1]ririnsuharsanti@stifar.ac.id*
[2]subagusw_fa@ugm.ac.id,
[3]nunungyuniartiwidodo@gmail.com,
[4]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. Ethanolic extract of the Curcuma aeruginosa Roxb rhizome was separated using the solid-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 IC50 21.93 ± 3.39µg/mL, ABTS method was HF with IC50 24.56±1,03 µg/mL and FRAP method was IF with IC50 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
Suharsanti, Wahyuono, S., Yuniarti, N., & Puji, A. (2024). Antioxidant Activity and Pancreatic Lipase Inhibition of Curcuma aeruginosa Roxb Rhizome Fractions. *Jurnal Biodjati*, 9(2), 228-243.

Jurnal Biodjati 9(2):228-243,November 2024

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

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, offerring 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±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, TMTZ (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 (SiO2)-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 (SiO2 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% $FeCl_3$ 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 Bouchardat 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 $FeCl_3$ 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_2SO_4 (Libermann-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% $AlCl_3$, 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_{50} value, representing the concentration needed to scavenge 50% of the DPPH free radicals, was determined. The percentage

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

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 $FeCl_3 \cdot 6H_2O$ 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 2 mM $FeSO_4$ 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:

$$\% \text{ FRAP} = \frac{\text{Nilai FRAP senyawa}}{\text{Nilai FRAP } FeSO_4\ 100\%} \times 100\%$$

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 pNPB 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 pNPB 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 Fractination

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 $Rf > 0.50$, while ethyl acetate and ethanol fractions had more polar compounds, showing spots with $Rf \leq 0.50$. The IF fraction contained highly polar compounds that remained at the baseline ($Rf = 0$). Overlapping spots were noted in HF, EAF, and EF. The chromatographic patterns of the extracts and fractions are shown in Figure 1.

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

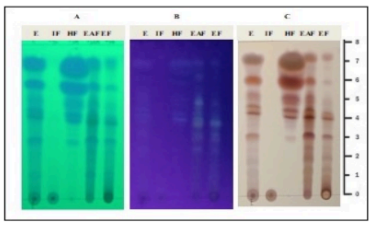

Figure 1. Thin layer chromatography results of Curcuma aeruginosa Roxb extracts and fractions. The spots are visualized using UV 254 nm (A), UV 366 nm (B), and cerium sulfate (C). E=extract, IF=insoluble frac\ tion, HF=n-hexane fraction, EAF=ethyl actate fraction, EF=ethanol fraction

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	+	+	+	+	+
Alkaloids	Mayer test	+	+	+	+	+
	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

Jurnal Biodjati 9(2):228-243,November 2024

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

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.

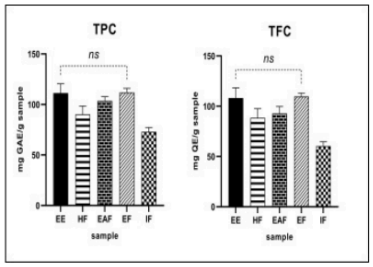

Figure 2. Total phenolics contents/TPCs (mg GAE/g sample) and total flavonoids contents/TFCs (mg QE/g sample) of ethanolic extract (EE), hexan fraction (HF), ethyl acetate fraction (EAF), ethanol fraction (EF), insoluble fraction (IF). Values are mean ± SD (n = 3), ns= not significant (α>0.05)

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 IC50 of 21.93 ± 3.39 µg/mL, while quercetin showed an IC50 of 14.90 ± 1.44 µg/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

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 IC50 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).

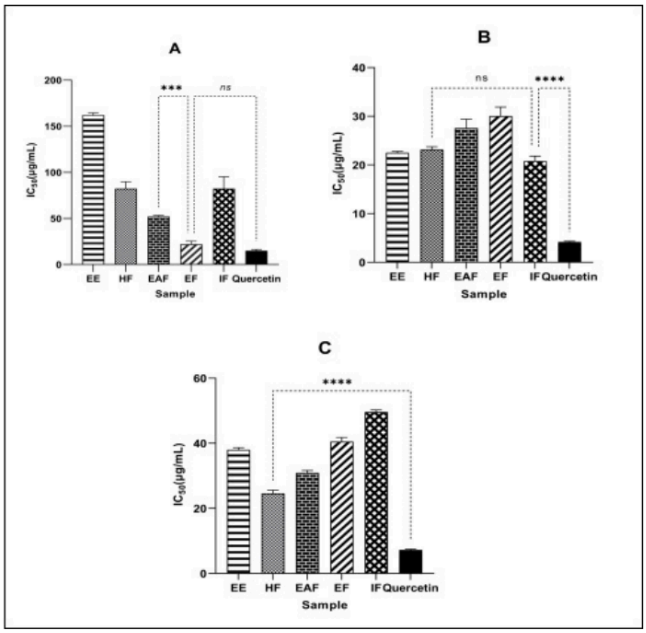

Figure 3. (A) DPPH radical scavenging activity, (B) FRAP (Ferric Reducing Antioxidant Power) activity, and (C) ABTS scavenging activity of ethanolic extract (EE), n-hexane fraction (HF), ethyl acetate fraction (EAF), ethanol fraction (EF), insoluble fraction (IF), and quercetin in IC50 (µg/mL). Values are mean ± SD (n = 3). *** and **** are significant ($\alpha<0.05$), ns = not significant ($\alpha>0.05$)

Jurnal Biodjati 9(2):228-243,November 2024

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

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-tripyridyltriazine to the complex ferro-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 ± 1.03 µg/mL, while EF had the lowest reduction/antioxidant activity, with an IC_{50} of 29.17 ± 1.57 µg/mL. TheIC_{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 IC50 of 23.12 ± 0.62 µg/mL, followed by EAF at 27.59 ± 1.82 µg/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 ± 1.03 µg/mL, followed by EAF at 30.91 ± 0.74 µg/mL, EF at 40.51 ± 1.26 µg/mL, and IF at 49.62 ± 0.6 µg/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 ± 0.24%, followed by EF at 29.66 ± 0.57%, IF at 27.03 ± 0.73%, and HF at 25.51 ± 4.14%. However, EAF's inhibition was lower than that of the positive control, orlistat, which had an inhibition rate of 46.07 ± 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 ± 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.

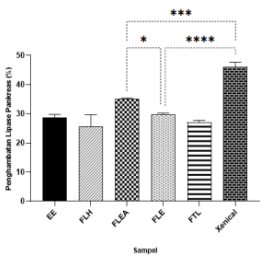

Figure 4. Pancreatic lipase Inhibitory effect (%) of ethanolic extract (EE), n-hexane fraction (HF), ethyl acetate fraction (EAF), ethanol fraction (EF), insoluble fraction (IF) and xenical as positive control. All groups were significantly different from the control ($\alpha<0.05$).

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 HRf 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 fractination and analysis process, **A.P,** and **Y.N** supervised the manuscript, and **S.R** collected and analyzed the data and wrote the manuscript.

ACKNOWLEDGMENTS

We would like to thanks Education Financial Services Center (Puslapdik) via the Indonesian Endowment Fund for Education (Lembaga Pengelola Dana Pendidikan (LPDP)) for the financial support

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Aberg, J. A., Lacy, C., Amstrong, L., Goldman, M., & Lance, L. L. (2009). *Drug Information Handbook 17th Edition*. American.

Alias, N., Leow, T. C., Shukuri, M., Ali, M., Noor, R., Raja, Z., & Rahman, A. (2017). Anti-obesity Potential of Selected Tropical Plants via Pancreatic Lipase Inhibition. *Advances in Obesity, Weight Management & Control*, 6(4) : 122–131. DOI: 10.15406/aowmc.2017.06.00163

Benzie, I. F. F., & Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Analytical Biochemistry*, 239(1) : 70–76. DOI: 10.1006/ABIO.1996.0292

Bonetti, C. I., Dalmagro, M., Friedrich, J. C., Jesus, D. R., Pinc, M. M., Alberton, O., & Lourenço, E. L. B. (2020). Phytochemical Profile and Evaluation of the Antimicrobial Activity of Echinodorus grandiflorus Crude Extract of the Leaves. *Journal of Agricultural Studies*, 8(4) : 176. DOI: 10.5296/JAS.V8I4.17339

Brown, W. V., Fujioka, K., Wilson, P. W. F., & Woodworth, K. A. (2009). Obesity: Why be concerned? American *Journal of Medicine*, 122(4A) : 4–11. DOI: 10.1016/j.amjmed.2009.01.002

Calabrone, L., Larocca, M., Marzocco, S., Martelli, G., & Rossano, R. (2015). Total Phenols and Flavonoids Content, Antioxidant Capacity and Lipase Inhibition of Root and Leaf Horseradish (*Armoracia rusticana*) Extracts. *Food and Nutrition Sciences*, 06(01) : 64–74. DOI: 10.4236/fns.2015.61008

De La Garza, A. L., Milagro, F. I., Boque, N., Campión, J., & Martínez, J. A. (2011). Natural inhibitors of pancreatic lipase as new players in obesity treatment. *Planta Medica*, 77(8) : 773–785. DOI: 10.1055/s-0030-1270924

Dilshad, R., & Batool, R. (2022). Phytochemical Analysis, Antibacterial Activity, Thin Layer Chromatography, Bacterial Efflux pump inhibition activity, Gas chromatography mass spectrometry. *Jordan Journal of Pharmaceutical Sciences*, 15(3) : 2022. DOI: 10.35516/jjps.v15i3.417

Faramayuda, F., Hermanto, F., Windyaswari, A. S., Riyanti, S., & Nurhayati, V. A. (2021). Identification of the Secondary Metabolites and Characterization of Lagerstroemia Loudonii T. & B. *Journal of Islamic Pharmacy*, 6(1) : 1–6. DOI: 10.18860/JIP.V6I1.11351

Fernández-Sánchez, A., Madrigal-Santillán, E., Bautista, M., Esquivel-Soto, J., Morales-González, Á., Esquivel-Chirino, C., Durante-Montiel, I., Sánchez-Rivera, G., Valadez-Vega, C., & Morales-González, J. A. (2011). Inflammation, oxidative stress, and obesity. *International Journal of Molecular Sciences*, 12(5) : 3117–3132. DOI: 10.3390/ijms12053117

Floegel, A., Kim, D. O., Chung, S. J., Koo, S. I., & Chun, O. K. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich

US foods. *Journal of Food Composition and Analysis*, 24(7) : 1043–1048. DOI: 10.1016/J.JFCA.2011.01.008

Galato, D., Ckless, K., Susin, M. F., Giacomelli, C., Ribeiro-do-Valle, R. M., & Spinelli, A. (2001). Antioxidant capacity of phenolic and related compounds: correlation among electrochemical, visible spectroscopy methods and structure-antioxidant activity. *Redox Report : Communications in Free Radical Research*, 6(4) : 243–250. DOI: 10.1179/135100001101536391

Gulua, L., Nikolaishvili, L., Jgenti, M., Turmanidze, T., & Dzneladze, G. (2018). Polyphenol content, anti-lipase and antioxidant activity of teas made in Georgia. *Annals of Agrarian Science*, 16(3) : 357–361. DOI: 10.1016/j.aasci.2018.06.006

Heck, A. M., Yanovski, J. A., & Calis, K. A. (2000). Orlistat, a new lipase inhibitor for the management of obesity. *Pharmacotherapy*, 20(3) : 270–279. DOI: 10.1592/phco.20.4.270.34882

Ilyasov, I. R., Beloborodov, V. L., Selivanova, I. A., & Terekhov, R. P. (2020). ABTS/PP Decolorization Assay of Antioxidant Capacity Reaction Pathways. International *Journal of Molecular Sciences*, 21(3) : 1-27. DOI: 10.3390/IJMS21031131

Jemal, K., Sandeep, B. V., & Pola, S. (2022). Phytochemical screening and in vitro antioxidant activity analysis of leaf and callus extracts of *Allophylus serratus* (Roxb) KURZ. *Jordan Journal of Pharmaceutical Sciences*, 15(1) : 51–69. DOI: 10.35516/JJPS.V15I1.291

Kemkes RI. (2018). Hasil Utama RISKESDAS 2018.

Kodjio, N., Atsafack, S., Fodouop, S., Kuiate, J.-R., & Gatsing, D. (2016). In vitro Antisalmonellal and Antioxidant Activities of Extracts and Fractions of *Curcuma longa* L. Rhizomes (*Zingiberaceae*). *International Journal of Biochemistry Research & Review*, 11(3) : 1–14. DOI: 10.9734/IJBCRR/2016/25106

Liu, T. T., Liu, X. T., Chen, Q. X., & Shi, Y. (2020). Lipase Inhibitors for Obesity: A Review. *Biomedicine and Pharmacotherapy*, 128 : 110314. DOI: 10.1016/j.biopha.2020.110314

Lobstein, T., Jackson-Leach, R., Powis, J., Brinsden, H., & Gray, M. (2023). World Obesity Atlas 2023. In World Obesity Federation (Issue March). www.johnclarksondesign.co.uk

Mardisiswojo, S., & Rajakmangunsudarso, H. (1985). Cabe puyang warisan nenek moyang. In Koleksi Buku UPT Perpustakaan Universitas Negeri Malang. Balai Pustaka.

Mauer, Y., Parker, M., & Kashyap, S. R. (2021). Antiobesity drug therapy: An individualized and comprehensive approach. *Cleveland Clinic Journal of Medicine*, 88(8) : 440–448. DOI: 10.3949/CCJM.88A.20080

Mukherjee, S., Pawar, N., Kulkarni, O., Nagarkar, B., Thopte, S., Bhujbal, A., & Pawar, P. (2011). Evaluation of free-radical quenching properties of standard Ayurvedic formulation Vayasthapana Rasayana. *BMC Complementary and Alternative Medicine*, 11(38) : 1-6. DOI: 10.1186/1472-6882-11-38

Nisar, T., Iqbal, M., Raza, A., Safdar, M., Iftikhar, F., & Waheed, M. (2015). Estimation of Total Phenolics and Free Radical Scavenging of Turmeric (*Curcuma longa*). *American-Eurasian J. Agric. & Environ*. Sci, 15(7) : 1272–1277. DOI: 10.5829/idosi.aejaes.2015.15.7.9527

Nurcholis, W., Khumaida, N., Syukur, M., & Bintang, M. (2016). Variability of total phenolic and flavonoid content and antioxidant activity among 20 *Curcuma aeruginosa* Roxb. Ac-

cessions of Indonesia. *Asian Journal of Biochemistry*, 11(3) : 142–148. DOI: 10.3923/ajb.2016.142.148

Nurcholis, W., Khumaida, N., Syukur, M., & Bintang, M. (2019). Variability of curcumin, demethoxycurcumin and bisdemethoxycurcumin contents in ethanolic extract from ten *Curcuma aeruginosa* roxb. Cultivated in West Java, Indonesia. *Asian Journal of Chemistry*, 31(11) : 2461–2465. DOI: /10.14233/ajchem.2019.22128

Nurcholis, W., Khumaida, N., Syukur, M., Bintang, M., & I. D.A.A.C., A. (2015). Phytochemical screening, antioxidant and cytotoxic activities in extracts of different rhizome parts from Curcuma aeruginosa RoxB. *International Journal of Research in Ayurveda and Pharmacy*, 6(5) : 634–637. DOI: 10.7897/2277-4343.065118

Permadi, A. (2008). *Membuat Kebun Tanaman Obat : Ciptakan Perkarangan Sehat Nan Asri (Cet. 1.)*. Pustaka Bunda.

Platzer, M., Kiese, S., Herfellner, T., Schweiggert-Weisz, U., Miesbauer, O., & Eisner, P. (2021). Common Trends and Differences in Antioxidant Activity Analysis of Phenolic Substances Using Single Electron Transfer Based Assays. *Molecules*, 26(5) : 1–17. DOI: 10.3390/molecules26051244

Pliego, J., Mateos, J. C., Rodriguez, J., Valero, F., Baeza, M., Femat, R., Camacho, R., Sandoval, G., & Herrera-López, E. J. (2015). Monitoring Lipase/Esterase Activity by Stopped Flow in a Sequential Injection Analysis System Using p-Nitrophenyl Butyrate. *Sensors* 2015, Vol. 15, Pages 2798-2811, 15(2) : 2798–2811. DOI: 10.3390/S150202798

Rahardhian, M. R. R., & Suharsanti, R. (2019). Potency Of Purification Extract From Belimbing Wuluh (*Averrhoa Bilimbi*) As Antioxidant And Anti-Tyrosinase. *Journal of Pharma Research*, 8(5) : 318–322. DOI: 10.5281/zenodo.3236703

Rahardhian, M. R. R., Suharsanti, R., Sugihartini, N., & Lukitaningsih, E. (2019). In Vitro Assessment of Total Phenolic , Total Flavonoid and Sunscreen Activities of Crude Ethanolic Extract of Belimbing wuluh (*Averrhoa bilimbi*) Fruits and Leaves. *Journal of Global Pharma Technology*, 11(04 (Suppl.)) : 308–313.

Rajurkar, N., & Hande, S. M. (2011). Estimation of Phytochemical Content and Antioxidant Activity of Some Selected Traditional Indian Medicinal Plants. *Indian Journal of Pharmaceutical Sciences*, 73(2) : 146. DOI: 10.4103/0250-474X.91574

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*, 26(9–10) : 1231–1237. DOI: 10.1016/S0891-5849(98)00315-3

Setiawan, V., Phangestu, S., Grace Soetikno, A., Arianti, A., & Kohar, I. (2021). Rapid screening analysis of antioxidant activities in green tea products using DPPH and FRAP. *Pharmaceutical Journal of Indonesia*, 7(1) : 9–14. DOI: 10.21776/UB.PJI.2021.007.01.2

Simoh, S., Sew, Y. S., Abd Rahim, F., Ahmad, M. A., & Zainal, A. (2018). Comparative Analysis of Metabolites and Antioxidant Potentials from Different Plant Parts of *Curcuma aeruginosa* Roxb. *Sains Malaysiana*, 47(12) : 3031–3041. DOI: 10.17576/jsm-2018-4712-13

Sosnowska, D., Podsędek, A., Kucharska, A. Z., Redzynia, M., Opęchowska, M., & Koziołkiewicz, M. (2016). Comparison of in vitro anti-lipase and antioxidant activities, and composition of commer-

cial chokeberry juices. *European Food Research and Technology*, 242(4) : 505–515. DOI: 10.1007/s00217-015-2561-4

Sudha, A., & Srinivasan, P. (2014). Bioassay-guided isolation and antioxidant evaluation of flavonoid compound from aerial parts of *Lippia nodiflora* L. *BioMed Research International*, 2014 : 1-10. DOI: 10.1155/2014/549836

Sugita, P., Octaviana, N., Wukirsari, T., & Rahayu, D. U. . (2018). Chemical constituent and antioxidant activity of methanol extract from Indonesian Curcuma aeruginosa roxb. rhizome. *Journal of Pharmacy Research*, 10(1) : 293–297.

Theafelicia, Z., & Wulan, S. N. (2023). Perbandingan Berbagai Metode Pengujian Aktivitas Antioksidan (DPPH, ABTS DAN FRAP) Pada Teh Hitam (*Camellia sinensis*). *Jurnal Teknologi Pertanian*, 24(1) : 35–44. DOI: 10.21776/ub.jtp.2023.024.01.4

Zhang, Y., Liu, J., Yao, J., Ji, G., Qian, L., Wang, J., Zhang, G., Tian, J., Nie, Y., Zhang, Y. E., Gold, M. S., & Liu, Y. (2014). Obesity: Pathophysiology and intervention. *Nutrients*, 6(11) : 5153–5183. DOI: 10.3390/nu6115153

Zohmachhuana, A., Malsawmdawngliana, Lalnunmawia, F., Mathipi, V., Lalrinzuali, K., & Kumar, N. S. (2022). *Curcuma aeruginosa* Roxb. Exhibits Cytotoxicity in A-549 and HeLa Cells by Inducing Apoptosis Through Caspase-Dependent Pathways. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, 150(113039) : 1–13. DOI: 10.1016/J.BIOPHA.2022.113039

Antioxidant Activity and Pancreatic Lipase Inhibition of Curcuma aeruginosa Roxb Rhizome Fractions

ORIGINALITY REPORT

13%	12%	10%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	dev.journal.ugm.ac.id Internet Source	4%
2	journal.uinsgd.ac.id Internet Source	4%
3	Whika Febria Dewatisari, Nelsiani To'bungan. "Phytochemical Constituent, Antibacterial and Antioxidant Leaves of Dracaena trifasciata (Prain) Mabb.", Jurnal Biodjati, 2024 Publication	1%
4	Ikha Safitri, Arie Antasari Kushadiwiojayanto, Mega Sari Juane Sofiana, Duc-Hung Nguyen, Rebiha Adjout. "Investigating Species Composition and Abundance of Marine Microalgae from Temajo Island Waters, West Kalimantan, Indonesia", Jurnal Biodjati, 2024 Publication	1%
5	www.myfoodresearch.com Internet Source	1%
6	www.wjpmr.com Internet Source	1%
7	Submitted to Fakultas Ekonomi dan Bisnis Universitas Gadjah Mada Student Paper	1%
8	Submitted to fpptijateng Student Paper	1%
9	www.ajbasweb.com Internet Source	1%

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		