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Characterization of Curcumin from *Curcuma purpurascens* Blume and Test of Its Activity as Antioxidant and Antilipase

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ABSTRACT

Curcuma purpurascens Blume is a plant in the family Zingiberaceae and known as temu blenyeh. This plant has been used as a medicinal plant but there is still little research and not much has been reported about its chemical components. The research aimed to isolate and characterize the chemical components of the rhizomes of *C. purpurascens* and test its activity as an antioxidant and antilipase. The ethanol extract of temu blenyeh (*C. purpurascens* Blume) underwent antioxidant activity-guided purification using vacuum liquid chromatography to obtain pure compounds. The antioxidant activity test used the DPPH (2,2-diphenyl-1picrylhydrazyl) method, while the antilipase activity was tested by pancreatic lipase inhibition assay. The antioxidant activity (IC_{50}) results showed that ascorbic acid (5.83±0.18µg/mL) was significantly more potent than EE (55.501±0.36µg/mL), EASF (44.46±0.28µg/mL), and isolate 1 (71.31±0.22µg/mL), whereas EASF was significantly more potent than isolate 1. The antilipase activity (IC_{50}) results showed that orlistat (9.23 ±0.75µg/mL) was significantly more potent than EE (117.86±10.72µg/mL), EASF (59.58 ±8.58µg/mL), and isolate 1 (54.79 ±5.59µg/mL), whereas there was no significant difference between EASF and isolate 1. Curcumin (yellow-orange powder form) was identified from the ethyl acetate fraction of *C. purpurascens* rhizomes as isolate 1 using UV-Vis, IR, UPLC-MSMS, 1D-NMR, and 2D-NMR analysis.

Keywords: antioxidant, antilipase, curcumin, *Curcuma purpurascens*

INTRODUCTION

Obesity is a chronic multifactorial disease and one of the main causes of morbidity and premature death in the world (Vangoori, *et al.,* 2019). The prevalence of obesity is estimated to increase by 10% in 2035 (Ralston and Baur, 2023). Meanwhile, the prevalence of obesity is reported to be increasing globally. In Indonesia, in 2018 the number of individuals in the overweight category was 13.6%, while the number of individuals with obesity was 21.8% (Riskesdas, 2018). The obesity category is assessed from the Body Mass Index (BMI) in adults; if there is an increase in BMI of >25 kg/m^2, then it is categorized as overweight and >30 kg/m^2 is categorized as obese (Riskesdas, 2018; Ralston & Baur, 2023; Vangoori *et al*., 2019). An increase in BMI, according to the World Health Organization (WHO), (2023) is the main trigger for degenerative and non-communicable diseases such as cardiovascular disease, osteoarthritis, cancer, joint disease, and diabetes. Hyperlipidemia can trigger an increase in oxidative stress in obesity cases. An increase in BMI in non-communicable diseases had an impact on the death rate of the world population that reached 38% in 2020 due to overweight and obesity (Ralston and Baur, 2023;

WHO, 2023). Therefore, it is necessary to make more efforts to prevent, manage, and treat obesity.

One of the efforts to treat obesity and overweight is by using plant-based medicines which serve as a herbal treatment strategy with few side effects and affordable costs (Marliyana *et al.*, 2018; Vangoori, *et al*., 2019). Currently, treatment of chronic diseases using medicinal plants is increasing globally. Medicinal plants have active compounds; some of them have been synthesized and used in modern medicine. Approximately 25% of medicines are made from plant extracts and used in modern medicine. Medicinal plants can be used directly or indirectly, such as through an extraction process, so they can produce new active compounds with pharmacological and therapeutic effects (Regina *et al.*, 2015; Khan *et al.*, 2017; Subositi and Wahyono, 2019).

Plants used in traditional medicine to treat various types of diseases are generally of the rhizome type, such as those in the family Zingiberaceae. One of the genera in this family is Curcuma (Marliyana, 2018). The genus Curcuma widely spread in Asia, Australia, and Africa, consisting of more than 47 genera and approximately 1000 species (Sasikumar, 2005; Ayati *et al.*, 2019; Atun *et al.*, 2020; Pramiastuti *et al.*, 2023). Rhizomes of the genus Curcuma have been reported to have various pharmacological activities, including as an antioxidant (Caiqin *et al.*, 2018; Atun *et al.*, 2020), antifungal, anticancer (Naksuriya *et al.*, 2014), antidiabetic (Kato *et al.*, 2016), antivirus, antimicrobial (Moran *et al.*, 2016; Atun *et al.*, 2020), anti-inflammatory (Yuan *et al.*, 2018), antiartherosclerotic, antiaging, antiarthritic, antidepressant (Nelson *et al.*, 2017), antiobesity (Alias *et al.*, 2017; Subositi & Wahyono, 2019) antihepatotoxic and antiproliferative agent (Srivastava, 2006; Policegoudra *et al.*, 2010; Naksuriya *et al.*, 2014; Padalia *et al.*, 2014; Jeon *et al.*, 2015; Kato *et al.*, 2016; Nelson *et al.*, 2017; Yuan *et al.*, 2018; Diastuti, Asnani and Chasani, 2019). *C. purpurascens* Blume is a plant species native to Indonesia from the family Zingiberaceae and used in traditional medicine (Pramiastuti *et al.*, 2023).

Several studies have been carried out on the genus Curcuma such as the rhizomes of *C.amada*, *C.longa*, *C.xanthorrhiza, C.domestica, Caeruginosa, C.zedoria, C.soloensis,* and *C.heynaena* (Cucuzza *et al.*, 2008; Atun *et al.*, 2020). Secondary metabolites isolated from the genus Curcuma are the curcuminoid group consisting of curcumin, desmetoxycurcumin, and bidesmetoxycurcumin (Lateef *et al*., 2016; Hamdi, 2015; Marliyana *et al.*, 2018; Pramiastuti *et al.*, 2023; Vitasari, 2016; Wikara *et al.*, 2016). Meanwhile, the monoterpene group includes 1.8-cineol, thymol, borneol, and p-cymen-8-ol, while the sesquiterpene group includes ar-curcumene, curcuminol (Halim *et al.*, 2012; Hong *et al.*, 2014) turmerone, ar-turmeron, β-seskuifelandren (Hong *et al.*, 2014; Lateef *et al.*, 2016), curlone, germacrone, curzerene, turmerone (Rouhollahi, *et al.*, 2014; Simoh and Zainal, 2015; Theanphong, 2015), and xanthorrhizol (Jantan *et al.*, 2012; Mangunwardoyo *et al*., 2012).

Temu blenyeh (*C. purpurascens*) is a species from the genus Curcuma that is still rarely explored and not much has been reported about its secondary metabolite content (Babu *et al.*, 2016). Morphologically and chemotaxonomically, the rhizome of temu blenyeh resembles that of turmeric (*C. longa*) with a similarity level of 75% (Setyawan, 2003; Pramiastuti *et al.*, 2023). The light or pale yellow rhizomes of temu blenyeh have a distinctive aroma like ginger (Hong *et al.*, 2014; Rouhollahi, 2016). Empirically, temu blenyeh is used to treat skin disease, boil, fever, and wound. Several studies on the pharmacological effects of *C. purpurascens* show antioxidant, gastroprotective, antimicrobial, antifungal, cytotoxic, antiproliferative, hepatoprotective, anticancer, and anti-inflammatory activities (Hamdi, 2015; Jalip *et al*., 2013; Pramiastuti *et al*., 2023; Rouhollahi *et al*., 2015; Rouhollahi, 2016; Rouhollahi *et al*., 2014; Sinaga *et al.*, 2018 ; Suprihatin *et al*., 2020).

Despite the therapeutic use and medicinal properties of *C. purpurascens*, there have been no reports on its bioactive molecules. This research aimed to investigate its antioxidant and antilipase potential by inhibiting pancreatic lipase from temu blenyeh in vitro, as well as isolating and characterizing the compounds. The results of this research can add to the database of compounds in *C. purpurascens* Blume which can then be used as a source of medicinal compounds.

MATERIALS AND METHODS

Temu blenyeh (*C. purpurascens* Blume) were obtained from UPTD Wisata Kesehatan Jamu Kalibakung (Herbal Health Tourism) Kalibakung Village RT 6, Balapulang Subdistrict, Tegal Regency, Central Java, Indonesia, and altitude 435 meters above sea level poscode: 52464, during October 2020. This plant had been validated by a botanist (Dr. Djoko Santosa), from the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada (UGM), Yogyakarta,

Indonesia (specimen number 14.12.1/UN1/FFA/BF/PT/2021).

All solvents and chemicals used were from Merck. Ethanol, n-hexane, methanol, ethyl acetate, chloroform, DMSO, Dichlormethane, 2,2-diphenyl-1picrylhydrazyl (DPPH, Sigma), Ascorbic Acid (Sigma), Orlistat (Nufarindo), 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), potassium phosphate buffer pH 7.2 (Sigma- Aldrich; St. Louis, MO, USA). All purchases of Sigma, Aldrich materials were done through PT. Kairos Indonesia suppliers.

Bropose Rotary Evaporator, Shimadzu UV-Vis spectrophotometry, Bruker Alpha FTIR, Angilent HPLC, Acquity UPLC-MSMS, Joel NMR spectrometer 400 MHz (1H-NMR) and 100 MHz (13C-NMr), Vacuum liquid chromatography (VLC) were carried out using Si -gel Merck 60 GF254, Germany, TLC analyzer on G/UV 254 seal plate 20x20 cm Nacharey Nagel Germany, TLC scanner.

Extraction and Isolation

C. purpurascens rhizome powder (500 g) was macerated with ethanol for 3 days with stirring every 24 hours; the filtrate was separated and the residue was re-macerated three times. The filtrate was collected and concentrated using a vacuum evaporator to obtain a concentrated extract.

The concentrated ethanol extract was then fractionated using the trituration method. 2.00 g of Ethanol Extract (EE) was triturated with n-hexane then vortexed for 10 minutes. Fractionation with n-hexane was carried out four times. The collected filtrate was evaporated using a rotary vacuum evaporator and called the n-hexane soluble fraction (HSF). The residue was successively partitioned with ethyl acetate and methanol in the same way, until the ethyl acetate soluble fraction (EASF), methanol soluble fraction (MSF), and methanol insoluble fraction (MIF) were obtained. The ethyl acetate soluble fraction (active fraction) was then used for isolation.

The ethyl acetate soluble fraction (20 g) was then fractionated by liquid vacuum chromatography (LVC) using the eluent n-hexane: ethyl acetate whose polarity increased in a gradient starting from 100:0 to 0:100 (v/v), ending with 100% methanol to produce 14 fractions categorized into 8 sub-fractions (SF a-h). Sub fraction b was proven to have the highest % inhibition against DPPH free radicals. The active sub-fraction (SF b) was then further separated by preparative thin layer chromatography using the eluent n-hexane : ethyl acetate (2:1) for 2x elution and produced 10 bands (SSF 1-10). Based on the results of the analysis using TLC, sub-sub fraction 8 (SSF8) still had to be further separated using PTLC with chloroform eluent: dichlormethane (1:2) to produce a pure isolate. The pure isolate (1) produced was a yellow-orange powder in sufficient quantities of 53.2 mg. The isolated compounds were then analysed using UV-Vis, IR, UPLC-MSMS and 1H NMR, and 2D NMR spectroscopy. Next, its antioxidant activity was tested using the DPPH and antilipase methods to inhibit pancreatic lipase.

Determination of Antioxidant Activity Using the DPPH Method

Antioxidant activity test used 2,2-diphenyl-1-picrylhydrazine (DPPH) reagent as a source of free radicals with modification (Pramiastuti *et al.*, 2021; Alekhya, 2022; Julianti, *et al*, 2022). A total of 100 µL of extract with various concentrations (2-100 µg/mL was added with 1.0 mL of 0.05 µM DPPH solution and up to 5.0 mL of methanol. The mixture was then vortexed and left for 30 minutes in a dark room, closed. The absorbance of the solution was then measured at a wavelength of 514.5 nm. The same thing was done for blank measurements (methanol). The results of the antioxidant activity test were compared with standard ascorbic acid. Each test was done in triplicate. Free radical scavenging activity is expressed as IC_{50} where the sample concentration can reduce 50% of DPPH free radicals. The smaller the IC_{50} value, the stronger the antioxidant activity (Policegoudra *et al.*, 2007; Purwanto *et al*., 2017). The inhibition percentage was calculated using the following formula:

$$\% \text{ inhibition} = \left[\frac{(AB-AA)}{AB}\right] \times 100 \quad \text{.....(1)}$$

AB = absorbance of the DPPH blank solution
AA = absorbance of the test solution

***In Vitro* Pancreatic Lipase Inhibition Assay**

The ability of a compound to inhibit PPL using a modified method of the method was described by (Alias *et al*., 2017; Kim *et al*., 2010). Porcine pancreatic lipase (PPL) activity was measured using the substrate p-nitropheyl butyrase (pNPB). 4 mM phosphate buffer solution (pH 7.2) and PNPB were made into stock solutions up to 10 mL. Porcine pancreatic lipase (PPL) solution was prepared just before use by dissolving 10 mg in 10 mL buffer (1 mg/mL). The

concentrations used for testing were 250, 125, 61.5, 31.25, and 15.6 µg/mL.

Lipase activity was measured using pNPB as a substrate which was hydrolyzed to p-nitrophenol at a wavelength of 405 nm using a UV-transparent 96-well plate on an ELISA Reader (Multimode reader Biotex Synergy HTX). To determine lipase inhibition needed 50 µL PPL solution, extract, ethyl acetate fraction, isolate, and orlistat (positive control) 50 µL each with varying concentrations of 250, 125, 61.5, 31.25 and 15.6 µg/mL was preincubated at 37 °C for 10 minutes. Next, 50 µL of pNPB substrate was added, all in a final volume of 150 µL and incubated at 37 °C for 10 minutes. The activity of DMSO as a negative control was also measured with and without inhibitors. Each test was carried out in triplicate. Inhibitory activity (I) was calculated using the following formula:

$$\text{Inhibitory activity (I\%)} = [100 - \frac{(B-b)}{A-a} \times 100] \quad .....(2)$$

A = activity without inhibitor; a= negative control without inhibitor; B= activity with inhibitor; b= negative control with inhibitor

Data Analysis

The experiment was done in triplicate and the data were expressed as mean ± standard deviation (SD). All statistical analyses were performed using a prism graph pad (version 9.1.2; Graph Pad Inc. software San Diego, CA, USA). IC_{50} value represents the concentration of the test sample causing 50% inhibition. Result of < 0.05 was considered significant.

RESULTS AND DISCUSSION

The chemical components of *C. purpurascens* Blume rhizomes were isolated by maceration with 96% ethanol solvent to obtain an ethanol extract followed by fractionation. Next, the active fraction was separated and purified using various chromatographic techniques such as VLC, PTLC and TLC to obtain pure isolates.

Extraction of temu blenyeh (500 g) produced 6.5 g of 96% ethanol extract (EE), and then fractionated using the trituration method using n-hexane, ethyl acetate, and methanol as solvents. The fractionation produced 4 fractions, namely HSF, EASF, MSF, and MISF. EE and EASF showed DPPH scavenging activity in the strong category using ascorbic acid as a positive control (figure 1). The DPPH scavenging from the ethyl acetate fraction was in the strong category, so it was chosen for the isolation of the bioactive compounds responsible for antioxidant and antilipase activity.

The active fraction (EASF) was then separated by vacuum liquid chromatography with a stationary phase using silica gel and a mobile phase in a gradient system using n-hexane: ethyl acetate 100:0 to 0:100 and ended with 100% methanol. The active fraction of the VLC results was SFb including the highest inhibition of DPPH free radicals at 93.62%. The SFb fraction was then purified by preparative thin layer chromatography with a mobile phase using n-hexane: ethyl acetate (2:1) to produce ten fractions (SSF1-SSF10). The eighth fraction (SFF8) showed the strongest DPPH scavenging activity of 69.23% (at a concentration of 100 ppm) (Figure 2). Next, the SSF8 fraction was purified using preparative thin layer chromatography twice with a mobile phase using chloroform : dichloromethane (1:2) to produce a pure isolate (isolate 1). The antioxidant and antilipase activity was tested on the extract, ethyl acetate soluble fraction, and isolate 1. Isolate 1 had DPPH free radical scavenging activity of 59.7% at a concentration of 100 µg/mL. The purity of isolate 1 as a bioactive antioxidant and antilipase compound was detected by TLC using 5 mobile phases with different polarities show one spot (Figure 1).

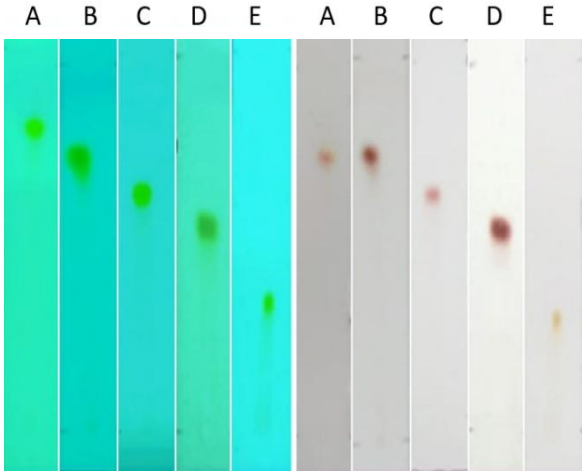

Figure 1. Purity of Isolates by TLC

The isolate purity was confirmed using HPLC, showing a single peak at 423 nm with a retention time of 3.902 minutes with a purity of 99.61% (Supplementary Figure 1A). The bioactive compound (isolate 1) was in the form of orange powder weighing 0.018 g (14.4%). Next, the isolated compounds were analysed using spectroscopy to confirm their structure (Figure 2).

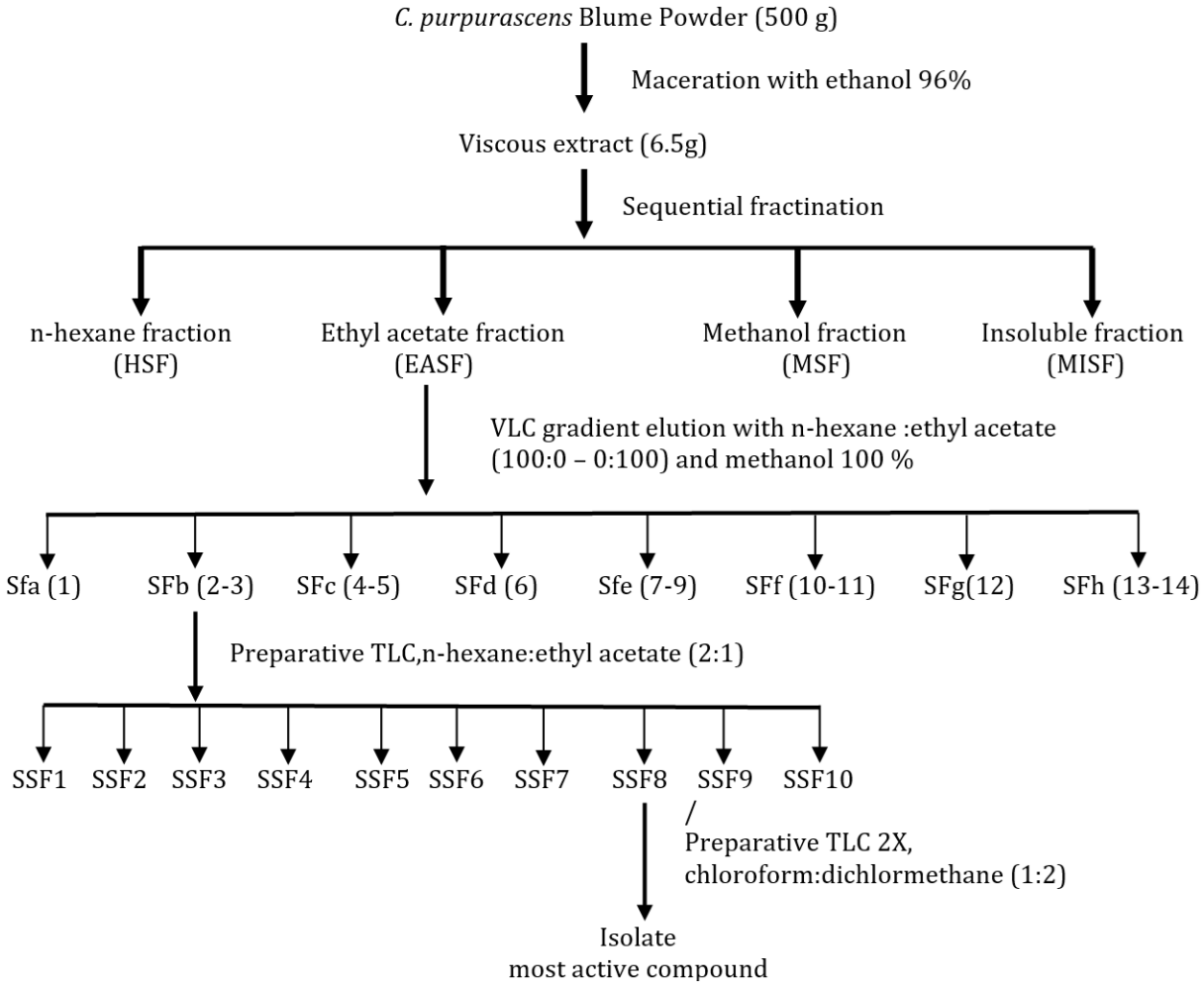

Figure 2. Bioassay-guided isolation of Curcumin from *C. purpurascens* Blume rhizome

The structures of the bioactive compounds were elucidated by analysing data obtained from various spectroscopies, including FTIR, UPLCMSMS, ^{1}HNMR, and ^{13}CNMR (Table I).

The components of the soluble fraction of ethyl acetate were isolated by chromatograpy (Atun *et al.*, 2020; Zahran *et al.*, 2020); melting point 170.4 -171 °C (isolate 1) which is in accordance with the literature mp = 181-182 °C (Li *et al.*, 2009; Ahmed *et al.*, 2017), 180-181 °C (Zahran *et al.*, 2020). The UV spectrum data of isolate 1 shows that there are two maximum wavelength peaks at 220.5 nm and 423 nm (Supplementary Figure 1B) which are similar to the UV spectrum of curcumin in methanol at wavelengths of 262 nm and 424 nm (Subhan *et al.*, 2013) with a yellow orange powder (Atun *et al.*, 2020). The IR spectrum data showed the presence of a strong OH (hydroxy group) at the wave number 3324 cm^{-1}, CH_2 and CH_3 asymmetric stretching at 2942 and 2834 cm^{-1}, absorption of the carbonyl group (C=O) at 1626 cm^{-1}, a C=C group conjugated to C=O at the wave number of 1583 cm^{-1}, the absorption of a benzene ring at at 1511 cm^{-1}, the CO enol group at the peak of 1445 cm^{-1}, a flexible CH_3 group at the wave number 1365 cm^{-1}, and a CO phenolic group at 1253 cm^{-1}, the COC group in OCH3 at an absorption of 1021 cm^{-1}, two adjacent aromatic CH_s at the wave numbers of 844 and 803 cm^{-1}, while an aromatic CH group at 713 cm^{-1}. The IR spectrum data explained that the compound had a carbonyl group conjugated with a benzene ring (aromatic). The IR spectrum of isolate 1 was shown in (Supplementary Figure 2A). The FTIR identification results of isolate 1 were the same as the curcumin spectrum results from other studies.

Table I. ^{1}H-NMR, ^{13}C-NMR and two-dimensional NMR data of the isolated compound (isolate 1) of Curcumin

No	Zahran *et al.,* 2020		Isolate (1)			
	δC(ppm)	δH (ΣH, mult., J in Hz)	δC (ppm)	δH (ΣH, mult., J in Hz)	HMBC	COZY
1	100.93	6.05 (1H, s, enol-form)	101.35	6.02 (1H, s)	C-2.2', C-3.3'	-
2.2'	183.25	-	183.75	-	-	-
2'-OH	-	9.68(2H, br-s)	-	10.00 (1H, s)		-
3.3'	121.11	6.75(2H,d)	121.63	6.72 (2H, d, 15.84 Hz)	C-1, C-2.2', C-5.5'	H-4.4'
4.4'	140.77	7.53 (2H,d)	141.25	7.50 (2H, d, 15.88 Hz)	C-2,2', C-3,3', C-5.5', C-6.6', C-10.10'	H-3.3'
5.5'	126.37	-	126.87	-	-	-
6.6'	111.31	7.31(2H,d)	111.88	7.28 (2H, d, 1.96 Hz)	C-4.4', C-5.5', C-7.7', C-8.8', C-10.10'	-
7.7'	148.02	-	148.53	-	-	-
7,7'-OCH3	55.71	3.83(6H,s)	56.22	3.80 (6H, s)	C-7.7'	-
8.8'	149.37	-	149.89	-	-	-
8.8'-OH	-	-	-	9.61 (2H, s)	C-7.7', C-8.8', C-9.9'	-
9.9'	115.73	6.80 (2H,d)	116.23	6.78 (2H, d, 8.2 Hz)	C-5.5', C-7.7', C-8.8'	H-9.9'
10.10'	123.19	7.13(2H, dd)	123.67	7.11 (2H, dd, 2.0, 8.28 Hz)	C-4.4', C-6.6', C-8.8'	H-10.10'

Curcumin had a spectrum of vmax cm-^{1}: 3324, 2942, 2834, 1626 and 1511 (Nandiyanto *et al.*, 2017; Zahran *et al.*, 2020).

^{1}H-NMR spectrum (500 MHz, DMSO-d6) showed a signal of one proton of the enol form at chemical shear (δH) 6.02 ppm (s,1H), one proton signal at δH 10.00 ppm (s, 1H) bound to hydroxy group. The methine proton signal bound to the C atom of alkene in the δH 6.72ppm (d,2H, 15.84 Hz) and δH 7.50 (d,2H,15.88Hz) regions formed a trans configuration; one aromatic meta coupling at δH 7.28 (d,2H, 1.96Hz), six methoxy protons were present at a chemical shift of δH 3.80 (s,2H), two hydroxyl protons on the aromatic ring were present at δH 9.61 (s ,2H), two aromatic ortho coupling pairs appeared at chemical shifts δH 6.78(d,2H, 8.2Hz) and 7.11 (dd,2H, 2.0, 8.28Hz) (Supplementary Figure 3A). Next data ^{13}C-NMR (125 MHz, DMSO-d6) spectrum described the presence of two symmetric carbonyls at δC 183.75 ppm (C2,C2'), twelve aromatic C atoms at δC 126.87, 111.88, 148.53, 149.89, 116.23, 123.67 ppm (C5,C5; C6,C6'; C7, C7'; C8,C8'; C9,C9'; C10,C10'), four alkene carbon atoms at δC 121.63 ppm (C3,C3') and 141.25 ppm (C4,C4'), two methoxyl carbons at δC 56.22 ppm (C7,C7'), one carbon of methylene/methine at δC 101.35 ppm (C1)(Zahran *et al.*, 2020) (Supplementary Figure 3B).

Overall, the position of each C and H atom in this compound was confirmed by HMBC experiments. Based on the HMBC data, there was an enol form of long-range correlation between H-1 and C-2,2' and C-3,3' (Supplementary Figure 4). The position of the enol form can be seen from the dynamic carbonyl group and hydroxy group exchanging positions at C-2 and C-2'. Curcumin has keto-enol tautomerism (Istyastono *et al.*, 2003; Malik and Mukherjee, 2014; Suharsanti *et al.*, 2023). Tautomerism is a rapid interconversion of keto and enol forms. Previous research conveyed that curcumin tends to be found in the enol form (Istyastono *et al.*, 2003; Malik and Mukherjee, 2014). The correlation of -OCH3 with C-7.7' confirmed that the methoxy position was directly linked to the quaternary carbon C-7.7'. The two symmetric aromatics in this compound were formed from 14 carbons, 4 oxygen, and 14 hydrogens to form 3-methoxy-2-hydroxy benzene, also confirmed by HMBC. From the negative mode MS data, we obtained [M – H]- m/z 367.18 meaning that the actual molecular weight was m/z 368

according to the molecular formula $C_{21}H_{20}O_6$ (Supplementary Figure 2B). Based on comparison with the reference, it was concluded that the compound was curcumin (trans, trans-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) (Zahran *et al.*, 2020).

Antioxidant activity by scavenging DPPH free radicals

DPPH (1,1-Diphenyl-2-picrylhydrazyl) is a red stable free radical with a maximum absorption at 517 nm. DPPH will be yellow if free radicals have been lost or wasted; this is used to evaluate free radical scavenging. DPPH free radicals will reduce when reacted with antioxidants because they donate hydrogen atoms. Thus, DPPH will change to diphenyl-picryl hydrazine, marked by a change in color from purple to yellow (Yokozawa *et al.*, 1998; Purwanti *et al.*, 2019; Vangoori, *et al.*, 2019).

The antioxidant activity of extracts, fractions and isolates was determined using the DPPH scavenging assay. DPPH scavenging activity was expressed in % inhibition and absorbance. The absorbance and percent inhibition data were then converted into a regression curve and a graph was obtained with the regression equation Y=0.3645x+29.780, with a value of r^2 = 0.9974 for the extract. The linear regression equation for EASF is Y= 0.2979x+36.746 with a value of r^2 = 0.9148, while the linear regression equation for isolate 1, Y = 0.3662X+23.880 with a value of r^2 = 0.9925. Meanwhile, the linear regression equation Y= 10.135x+11.315 with r^{tabel} = 0.9997 for the positive control, namely ascorbic acid. Furthermore, the antioxidant activity of the extract (EE), the ethyl acetate soluble fraction (EASF), isolate 1, and ascorbic acid were calculated for their IC_{50} values (Figure 3). When compared to previous research, *C. purpurascens* had varied antioxidant activity with IC_{50} values of 36-100 µg/mL (Jalip *et al.*, 2013; Sinaga *et al.*, 2018; Pramiastuti *et al.*, 2021). Tukey test data for antioxidant activity showed that ascorbic acid was significantly more potent than EE and EASF (*p value* <0,001). Ascorbic acid was also significantly more potent than isolate 1 (*p value*<0,0001). EASF was significantly more potent than isolate 1 (*p value*<0,001).

According to Jalip *et al.*, (2013), *C. purpurascens* had the strongest antioxidant activity compared to *C. aeruginosa*, *C. heynena*, *C. mango,* and *C.phaeocaulis*. Other promising sources of natural antioxidants from the genus Curcuma include *C.xanthoriza*, *C.longa*, *C.zedoria*, *C.amada,* and *C.aromatica* (Akter *et al.*, 2019). In several studies, plants in the genus Curcuma have been proven to contain bioactive compounds which have pharmacological effects, including antimicrobial, anticancer, anti-inflammatory, antioxidant, antihepototoxic, gastroprotective, antihyper-uricemic, antidiabetic effect (Rajkumari and Sanatombi, 2018). EASF is the most active fraction possible because many hydroxy groups are distributed in ethyl acetate (Alawiyah and Senania, 2022). Other research conducted by Kodjio *et al* (2016) showed that the ethyl acetate fraction of *C. longa* had greater antioxidant activity than the n-hexane fraction.

Curcumin is a polyphenolic compound that has therapeutic effects; one of which is through its antioxidant function. Curcumin has a phenolic hydroxyl group as an active site which is sterically inhibited by two methyl groups at the ortho position in the aromatic ring, so curcumin has antioxidant potential (Jovanovic *et al.*, 1999; Malik and Mukherjee, 2014).

Antilipase activity by inhibiting the pancreatic lipase enzyme

Antilipase activity was tested at a concentration of 125 µg/mL for PPL inhibition. Among the extracts, fractions, and isolates of *C. purpurascens* at a concentration of 125µg/mL, the one with the highest inhibition against PPL was the ethyl acetate soluble fraction (70.33%) and was close to Orlistat (73.60%). Many hydroxy groups such as in flavonoids and phenolic compounds are distributed in ethyl acetate, so the ethyl acetate fraction had stronger activity than the crude extract (Nuri *et al.*, 2020; Alawiyah and Senania, 2022). Phenolic compounds are able to form complexes with enzymes by non-specific bonds on the enzyme surface (Katz, Doughty and Ali, 2011; Nuri *et al.*, 2020). Curcumin isolate also had a phenol group which provides an antilipase effect with 61.28% inhibition. Orlistat as a positive control is known for anti-obesity treatment and a hydrogenated derivative of lipstatin which is able to inhibit the pancreatic lipase enzyme for a long time (Vangoori *et al.*, 2019). Orlistat is also an irreversible lipase inhibitor that binds to Serine 152 of the lipase covalently (Hadváry *et al.*, 1991; Vangoori, Dakshinamoorthi and Kavimani, 2019). The antioxidant activity of the isolate was not better than the extract and positive control but was still classified as strong activity (Blois, 1958).

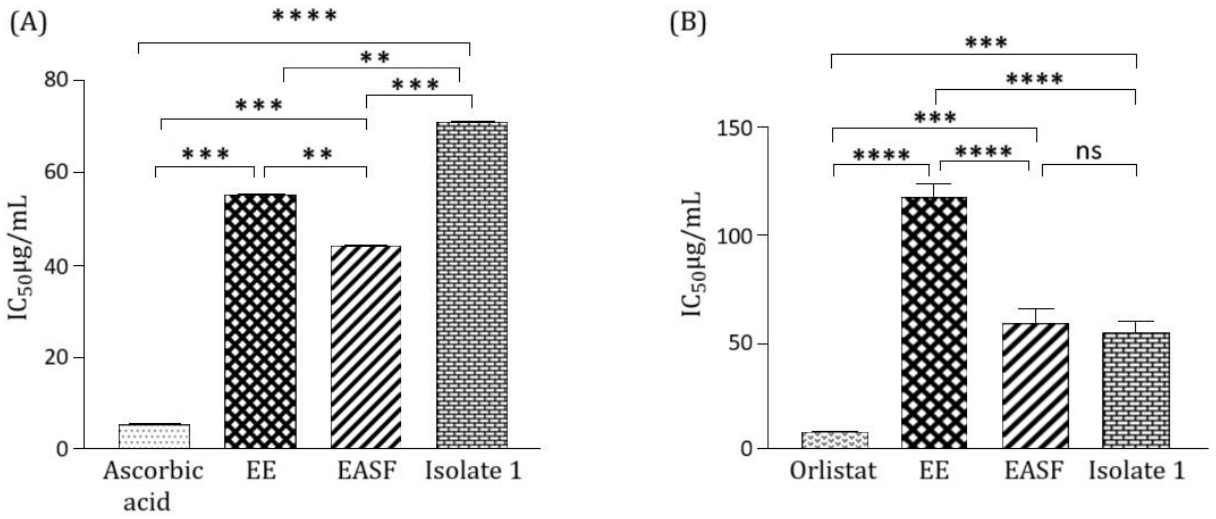

Figure 3. Antioxidant (A) and Antilipase (B) activity of *C. purpurascens* Blume rhizome Extract (EE), Ethyl acetate fraction (EASF), isolate, and positive control. Ns = not significant, ** sig (p<0.01), *** sig (p<0.001), **** sig (p<0.0001), n=3.

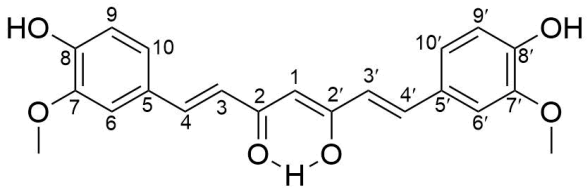

Figure 4. Structure of isolate 1 (curcumin)

Tukey test data for antilipase activity showed that orlistat was significantly more potent than EE (p value <0.0001) (Figure 3). Orlistat was also significantly more potent than EASF and isolate 1 (p value <0.001), whereas there was no significant difference (ns) between EASF and isolate 1. The isolate actually had better antilipase activity than the extract and fraction. In other words, the compound had a role in antilipase activity.

Pancreatic lipase is a lipolytic enzyme capable of hydrolyzing 50-70% of total fat, which works to convert triglyceride substrates into monoglycerides and free fatty acids (Liu *et al.*, 2020; Seyedan *et al*., 2015; Vangoori *et al*., 2019). Obesity is caused by a high amount of monoglycerides and free fatty acids in the body, the absorption of which can be slowed down by inhibiting the pancreatic lipase enzyme (Hidayat *et al*., 2014; Vangoori *et al.*, 2019). Orlistat was used as a positive control because it is an irreversible lipase inhibitor that binds covalently to Serine 152 of the lipase (Vangoori *et al.*, 2019). Obesity is the formation of complex compounds that are triggered by the presence of excessive fat and hypoxia in adipose tissue. This condition can trigger adipocytes to produce ROS, resulting in oxidative stress. This leads to adipocyte differentiation and fat accumulation (Utami *et al.*, 2019). Antioxidant and antilipase activities work synergistically in preventing obesity because antioxidant activity prevents oxidative stress and antilipase can reduce fat absorption. The results of this study provide information on the use of *C. purpurascens* Blume rhizomes in herbal medicine for the management of obesity. Therefore, the rhizome of this plant can be used as an alternative or complement in treating obesity comorbidities. Apart from that, the rhizome of this plant can also be used as a raw material for developing obesity drugs in the future. *C. purpurascens* rhizomes provide antioxidant and antilipase effects.

CONCLUSION

The biological activity of the extracts and rhizome fractions of *C. purpurascens* Blume was investigated. Ethanol extract (EE) and ethyl acetate soluble fraction (EASF) have DPPH free radical scavenging activity and pancreatic lipase inhibition. Of the several fractions, the ethyl acetate soluble fraction provides the best free radical scavenging activity and pancreatic lipase inhibition. The SSF8 component was successfully isolated as curcumin (enol form) and has DPPH free radical scavenging activity and pancreatic lipase inhibition. The *C. purpuracens* plant can be used to treat obesity by reducing oxidative stress and antilipase activity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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