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Isolation and Characterization of Curcumenotone, a Sesquiterpene from *Curcuma aeruginosa* Roxb as Antioxidant

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ABSTRACT

Antioxidant compounds are necessary to block the initiation of oxidation chain reactions and inhibit the formation of ROS which are the cause of various diseases. Natural antioxidants can be isolated from various sources: one of which is *Curcuma aeruginosa* Roxb Rhizome. This study was aimed to isolate and identify the antioxidant compounds from *C. aeruginosa* Roxb rhizome. To isolate the active compound, the 2,2-diphenyl-1-picrylhidrazyl (DPPH) free radical scavenging activity guide was used. Initially the *C. aeruginosa* Roxb rhizome powder was macerated with 70% ethanol to produce ethanol extract (EE). The EE was then fractionated gradually by increasing the polarity of solvents to produce n-hexane (HF), ethyl acetate (EAF), ethanol (EF) and insoluble (IF) fractions. EF as the active fraction, was fractionated into 6 fractions (EF.a-f) based on their TLC images. Two compounds (EF.a1 and EF.a2) were isolated from the active fraction (EF.a) by preparative TLC. EF.a2 which appeared as white crystals demonstrated the best activity as DPPH anti radical, having 96.24% purity (HPLC). EF.a2 appeared as a single peak in GC (Rt 12,78), and had a molecular weight of m/z 234. There were 2 absorbtion peaks at 226 nm and 260 nm in Uv-Vis spectrophotometer and absorption bands at 3364, 3314, 2905 and 1653 cm^{-1} on the FT-IR spectrum. Based on the spectroscopic data (^{1}H-,^{13}C-NMR) and the reported data, the EF.a2 was identified as sesquiterpene, curcumenotone ($C_{15}H_{22}O_2$). The curcumenotone was shown to be a potent antioxidant in the DPPH radical scavenging assay with an IC_{50} of 53.24±1.51 µg/mL, ABTS radical scavenging assay with an IC_{50} of 41.33±3.15 µg/mL, and FRAP assay with an IC_{50} of 37,82±2.02 µg/mL.

Keywords: Bioassay-Guided Isolation, *C. aeruginosa*, Antioxidant, Sesquiterpenes, Curcumenotone

INTRODUCTION

Reactive oxygen species (ROS) are inhibited by antioxidant compounds which works by blocking the initiation of the oxidation chain reaction (Mistriyani *et al*., 2021). Damage to biological molecules such as proteins, lipids, and nucleic acids caused by excess ROS can cause ischemia, atherosclerosis, arthritis, multi-tissue reperfusion injury, neurodegenerative disorders, cardiovascular disease, cancer, and AIDS (Patel *et al*., 2018). Natural antioxidants can be isolated from various sources one of which is from *Curcuma aeruginosa* Roxb Rhizome. Therefore, the development and utilization of antioxidants that are more effective, inexpensive, and natural are urgent. Bioassay-guided isolation is a strategy used to find active compounds that have certain biological activities. The main components of *C.*

aeruginosa Roxb identified in water distillation included champor (29.39%) and germacrone (21.21%) (Akarchariya *et al.*, 2017), β-pinene and 1,8 cineol as monoterpenes (21.47%) (Srivilai *et al.*, 2017), 1,8-cineol (22.65%), and germacrone (17.70%) (Srivilai, *et al.*, 2018), tropolene (18.1%) and eucalyptol (17.9%) (Fitria *et al.*, 2019), neocurdione (16.1%), curcumol (15.2%) and β-pinene (21.9%) (Pham *et al.*, 2018). The chromatographic separation of the compounds in *C. aeruginosa* Roxb extract includes curcumenol, isocurcumenol, germacrone, zederone, zedoarondiol, and dehydrocurdione (Suphrom *et al.*, 2012), dehydrocurdione, curcumenol, and germacrone (Srivilai *et al.*, 2018); pyrocurzerenone, dehydrochromolaenin, curzeone, linderazulene, curzerenone, 8, 12 - Epoxy - 1 (10), 4(15), 7, 11 -germacratetraen-6-one (Boutsada *et al.*, 2018); aeruginon and curcumenon (Atun *et al.*, 2016); and flavones (Hastuti *et al.*, 2016). Several compounds that have been isolated from *C. aeruginosa* Roxb isolate have been reported to have various activities: one of which is germacrone as antiandrogenic (Suphrom *et al.*, 2012), hair growth promoter (Srivilai *et al.*, 2018), antinociceptive (Hossain *et al.*, 2015), and anticancer (Atun *et al.*, 2016). In previous studies, the active fraction of antioxidants purified by GC-MS obtained two peaks at 8.19 and 12.29 minutes, and identified to be isoflavones (chromene) and sesquiterpene derivatives. No further separation or isolation of pure compounds was carried out from this purified fraction. This purified fraction has an antioxidant activity against the 2,2-diphenyl-1-picrylhidrazyl test with half-maximum inhibitory concentration > 1000 ppm (Sugita *et al.*, 2018). Therefore, this study isolated pure compounds with the highest antioxidant activity from *C. aeruginosa* Roxb rhizome using bioassay-guided isolation and to find out the names of the compounds.

MATERIALS AND METHODS

The materials used in this research were *C. aeruginosa* Roxb rhizomes (collection number: CA-01-03-20) which were collected from the Research and Development Center for Medicinal and Traditional Medicinal Plants, Tawangmangu, Central Java, Indonesia. The rhizomes were sun- and oven, then stored at a room temperature. This research also used ethanol, methanol, n-hexane, ethyl acetate, chloroform, glacial acetic acid and dichlormethane solvents pro analysis (Smart Lab Indonesia), Silica gel plate 60 F 20x20 cm (Merck, Germany), silica gel 60 F 254 nm for thin layer chromatography (Merck, Germany), cerium sulfate. Quercetin, DPPH, TPTZ, and ABTS (sigma-Aldrich St Louis, MO, 101 USA). The instruments for the structural elucidation were spectrophotometer UV-Vis (Shimadzu), FT-IR (Agilent technologies Cary 630) spectrometer, HPLC waters e2695 separations module, detector waters 2998 photodiode array detector, C18 sunfire column 4.6x150mm, GC-MS (simadzu QP2010 SE), ^{1}H-NMR (Jeol Resonance 400MHz), ^{13}C-NMR and APT (Jeol Resonance 100 MHz).

Extraction, Fractionation and Isolation

Dried *C. aeruginosa* Roxb rhizome was powdered and then 1 kg was extracted by re-maceration method using. The antioxidant activity and toxicity of *C. aeruginosa* rhizome extracted with 70% ethanol had better results than those extracted with 96% ethanol (Nurcholis *et al.*, 2015). A rotary vacuum evaporator was used at 60°C, 100 rpm until the extract was thick. The ethanol extract (EE) was partitioned by solid-liquid fractionation using non-polar (n-hexane), semi-polar (ethyl acetate), and polar (ethanol) solvents, subsequently. Fractionation was carried out by adding 100 mL of solvent to 30 g of extract, stirring with a magnetic stirrer for 5 min, and separating the fractions to obtain n-hexane soluble and insoluble fractions. The processes were repeated until the color of the solvent was clear. The n-hexane soluble fraction was collected, rotary vacuum evaporator was used at 60°C, 100 rpm until the fraction was thick and called the n-hexane soluble fraction (HF). The same process had been carried out to get the ethyl acetate and ethanol fractions. The fractionation resulted in n-hexane (HF), ethyl acetate (EAF), ethanol (EF), and insoluble (IF) fractions were obtained. The yield of the fractions was calculated. Based on previous research, the best scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was EF compared to other fractions.

EF, which had active antioxidant activity, was embedded on 60 F 254 silica gel powder at a ratio of 1:1. The separation was continued by vacuum liquid chromatography (VLC) with the gradient method starting from n-hexane : ethyl acetate 100:0 to 0:100 and finished with 100% methanol. A total of 12 VLC fractions labeled with EF1-12 were obtained and then identified by TLC using chloroform : ethanol : glacial acetic acid (94:5:1) as the mobile phase and the separated bands were visualized using UV 254 nm, 366 nm

and cerium sulfate. Fractions from VLC were categorized into 6 main fractions from 12 previous fractions based on thin layer chromatography (TLC) identification results namely EF.a : fraction number 1–3, EF.b: 4–6, EF.c: 7–9, EF.d : 10, EF.e : 11, EF.f : 12. The EF.a-f fraction was shown as the most active fraction based on the antioxidant test by the DPPH method.

The most active fraction, namely EF.a, was further separated by preparative thin layer chromatography using chloroform: dichlormethane (6:1) as the mobile phase. The separation using the mobile phase was carried out twice until a pure compound was obtained. The resulting isolated compounds were analyzed by TLC with chloroform : dichlormethane (6:1) as the mobile phase and sprayed with DPPH. Isolates 2 and 4 formed pale yellowish spots with a purple background. Fraction 2 was labeled EF.a1 while fraction 4 was labeled EF.a2. Then, the antioxidant activity od booth EF.a1 and EF.a2 isolates were tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH).

The purity of EF.a2 isolates, which were shown to be more active was then tested by TLC using 3 mobile phases and high performance liquid chromatography (HPLC) instruments. The structure of the EF.a2 was confirmed through interpretation of spectral data (UV, FT-IR, 1H NMR, 13C NMR, and MS). In addition, the antioxidant activity of EF.a2 was also tested by the FRAP and ABTS methods.

Antioxidant activity

DPPH Radical Scavenging Assay

The samples were dissolved in methanol and a certain concentration series was made. The antioxidant activity was determined by placing 0.2 mL of solution of each sample series in a test tube, followed by adding 4.0 mL of 0.1 mM DPPH for each concentration. The mixture was then homogenized by vortex stirring for 1min and left for 30 min as the operating time. The absorbance of the solution was read at a maximum wavelength of 517 nm. The same steps were carried out in measuring the quercetin standard series in an absorbance reader (Suharsanti *et al.*, 2019) (Rahardhian & Suharsanti 2019; Suharsanti *et al.*, 2019). The antioxidant activity of quercetin standard used as a comparator was prepared at a concentration of 10 µg/mL to 50 µg/mL. IC_{50} indicates the sample concentration required to capture 50% of the DPPH free radicals (Pourmorad *et al.*, 2002; Rahardhian & Suharsanti 2019). The absorbance of fractions and isolates from *C. aeruginosa* Roxb was calculated and expressed in the percentage (%) of antioxidant activity compared to the absorbance of the control using the formula:

$$\%\ antioxidant\ activity\ \frac{Abs.Control - Abs.Sample}{Abs.Control} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots (1)$$

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP solution was made by mixing 10 parts of an acetate buffer solution containing 0.1 M (PH 3.6), 1 part of a solution containing TPTZ (2,4,6-tripydyl-s-triazine) dissolved in 40 mM HCl, and 1 part of a solution containing FeCl3.6H2O 12 mM. In a flask containing 5 mL of methanol, a total of 5 mg of EF.a2 isolate was diluted. A certain concentration series was produced out of each individual solution. A test tube was given 0.2 mL of FRAP solution, and then 0.4 mL of solution was added to it for EF.a2 isolate to determine the antioxidant activity of the sample. After that, the mixture was homogenized by vortex stirring for 1 minute and left in a dark room for 10 minutes. The absorbance of the solution was measured at a maximum wavelength of 595.4 nm. A standard quercetin solution was prepared at a concentration ranging from 4 to 10 g/mL, and the absorbance of this solutions was also measured. Using the ferric ion reducing antioxidant power approach, the absorbance was used to determine the level of antioxidant activity. This result was referred to as FRAP. After that, the percentage of FRAP was computed by contrasting the FRAP value of the chemical with that of 12 mM FeSO4, which was regarded as 100% FRAP (Benzie & Strain, 1996; Setiawan *et al.*, 2021). The percentage calculation of the percentage of of the antioxidant activity was calculated using the following formula :

$$\%\ antioxidant\ activity = \frac{Compound\ FRAP\ value}{FeSO4FRAP\ value} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots (2)$$

ABTS Radical Scavenging Assay

A total of 5 mg of EF.a2 isolate was diluted with methanol in a 10 mL flask. From this solution, a certain concentration series was made. To determine the antioxidant activity, 4.0 mL of ABTS solution was poured into a test tube, and then 0.2 mL of EF.a2 isolate solution was added to the tube for each concentration. After that, the mixture was homogenized by vortex stirring for 1 min, and left in the dark for 6 minutes, as recommended by the

operating time. At a wavelength of 736 nm, the absorbance of the solution could be measured accurately. The same steps were carried out for measuring the quercetin standard series made with a concentration of 4 to 10g/mL. The absorbance of EF.a2 obtained was compared to the absorbance of the ABTS control to obtain the percentage of antioxidant activity (Mukherjee *et al.*, 2011; Rajurkar & Hande, 2011). The calculation used the following formula :

$$\% \text{ antioxidant activity } \frac{Abs.Control - Abs.Sample}{Abs.Control} \text{ x } 100\% \quad \ldots\ldots\ldots\ldots\ldots (3)$$

Data Analysis

The data were expressed as the mean ± standard deviation (SD) of experiments in triplicate. This statistical analysis in this study was carried out using a GraphPad Prism (version 9.1.2; Graph Pad Inc. software San Diego, CA, USA). IC_{50} value represented the concentration of the test sample causing 50% inhibition in which value <0.05 was considered significant.

RESULTS AND DISCUSSION

Antioxidant activity is tested using three different methods to determine the real antioxidant ability of a sample. A sample can be said to have an antioxidant activity if it has been tested on no less than two different types of antioxidant mechanisms. The DPPH method was used in this study because this method is simple, easy, fast and sensitive and only requires a small number of samples to evaluate the antioxidant activity of natural compounds, The test DPPH method has a radical scavenger mechanism. To complete the method with the same mechanism, ABTS was also used. FRAP was chosen to complete the antioxidant activity test because it has a reducing power mechanism. The DPPH method was used as an isolation guide. The test results revealed that the extracts and fractions had free radicals scavenging activity with an increasing concentration. EF was shown to have the highest activity IC_{50} of 21.93±3.39 µg/mL while quercetin had an IC_{50} of 14.90±1.44 µg/mL. There was no significant difference between the two after the statistical analysis was carried out. When compared with previous studies on the antioxidant activity of *C. aeruginosa* using the DPPH method in 20 accessions in the same country in Indonesia, the IC_{50} varied, ranging from 0.34 to 19.59% (Nurcholis *et al.*, 2016). In fact, the antioxidant activity is significantly influenced by the hydroxy phenolic compounds in the molecular structure. These compounds will react with free radicals, then form new radicals which are stabilized by the effect of aromatic nucleus resonance (Nisar *et al.*, 2015).

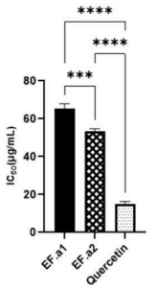

Figure 1. Free radical scavenging activity of EF.a1 and EF.a2 isolates with quercetin as standard, there were significant differences *** (sig 0.0004) and **** (sig <0.0001), n=3

EF was subjected to repeated bioassay-guided fractionation by VLC with a silica gel stationary phase and gradient system as a mobile phase, which started from n-hexane:ethyl acetate 100:0 to 0:100 and ended with 100% of methanol. Further purification of the active fraction obtained from VLC finally resulted in a bioactive antioxidant fraction from EF1-3 (EF.a), the mixture of n-hexane fractions: ethyl acetate (100:0, 90:10, and 80:20 v/v). The EF.a fraction was further purified using preparative thin layer chromatography twice with the mobile phase of chloroform: dichlormethane (6:1) to produce isolates EF.a1 and EF.a2 isolates. The antioxidant activity test using the DPPH method was carried out on both isolates (Figure 1). For the antioxidant activity test, DPPH which is an unstable nitrogen free radical compound (oxidant) will then be able to bind hydrogen ions derived from the test compound (antioxidant). The presence of antioxidant compounds from the extract and fraction samples changed the color in the DPPH solution in methanol from deep purple to yellow (Hossain *et al.*, 2015; Sugita *et al.*, 2018; Suharsanti *et al.*, 2019). The bioassay- guided isolation to obtain the active compound is schematically (Figure 3).

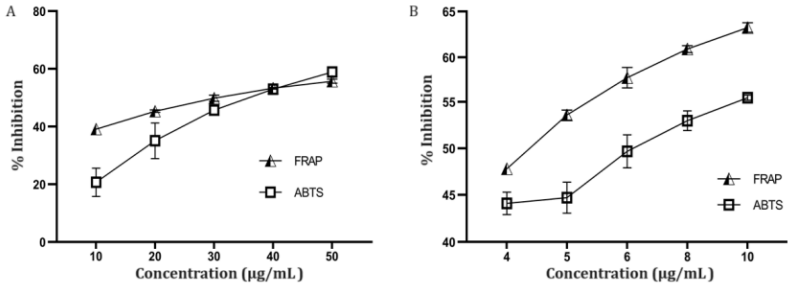

Figure 2. Antioxidant activity (% inhibition) of EF.a2 isolate (A) and quercetin (B) with FRAP and ABTS methods, n

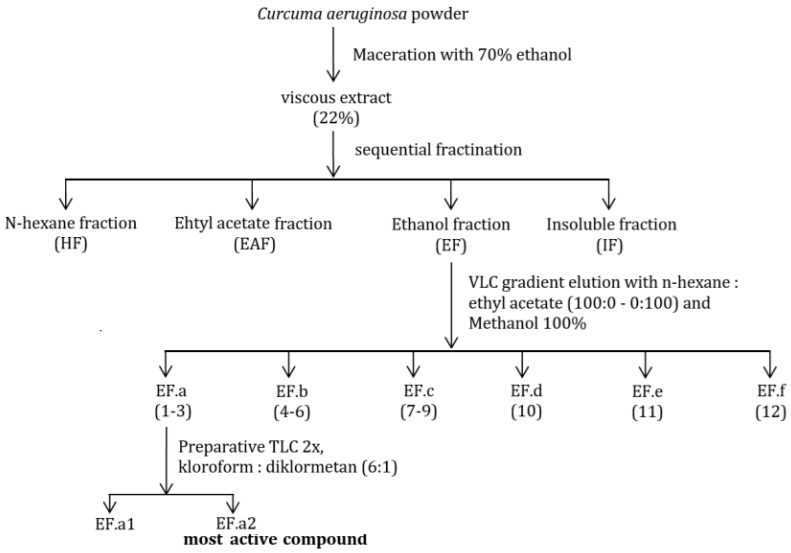

Figure 3. Bioassay- guided isolation of sesquiterpenes from *Curcuma aeruginosa* Roxb rhizome

Table I. Comparison of spectra data for ^{1}H-NMR and ^{13}C-NMR of EF.a2 isolate with other researchers

Carbon number	δc ppm						δH ppm					
			Curcumenol						Curcumenol			
	EF.a2 isolate	Curcumenotone (Zhang *et al.*, 2019)	**1** (Hamdi *et al.*, 2015)	**2** (Rahayu *et al.*, 2018)	**3** (Myint, 2006)	**4** (Firdaus, *et al.*, 2018)	EF.a2 isolate	Curcumenotone (Zhang *et al.*, 2019)	**1** (Hamdi *et al.*, 2015)	**2** (Rahayu *et al.*, 2018)	**3** (Myint, 2006)	**4** (Sugita, Firdaus, *et al.*, 2018)
1	51.2	52.4	51.3	51.4	51.2	51.25	2.3 (1H, m)	2.9 (1H, dd ,11.7; 8.3 Hz)	1.9 m	2.00 (1H,m,H-1)		1.96 (1H,m)
2	27.3	24.1	27.6	27.8	27.6	27.59	1.8 (2H, m)	α 1.77 (1H, m) β 1.98 (1H, m)	1.9/1.9 m	1.93 (4H,m,H-2, H-3)	1.54-2.00 (6H,m)	1.97 (2H,m)
3	31.2	30.1	31.2	31.4	31.3	31.22	1.9 (2H, m)	α 1.92 (1H, m) β 1.46 (1H, m)	1.9/1.9 m			1.90 (2H,m)
4	40.5	42.3	40.4	40.5	40.4	40.35	1.9 (1H, s)	1.92 (1H, q, 6.3 Hz)	2.62 d (15.6)	1.89 (1H,m,H-4)		1.91 (1H,m)
5	85.7	86.6	85.8	85.8	85.6	85.67	-	-	-	-	-	-
6	37.0	36.2	37.2	37.3	37.2	37.22	1.9 (2H, d, 1.2Hz)	α 1,93 (1H, d,14.7 Hz), β 3,03 (1H, d,14.7 Hz	2.11/2.66 d (15.4)	2.88 (1H,s,H6a), 2.66 (1H,d, 15.8 Hz)	2.11 (1H,brd, 15.4 Hz), 2.65 (1H,brd,154 Hz)	2.12 (1H,d,16.0 Hz), 2.66 (1H,d,16.0 Hz)
7	141.2	141.5	139.2	139.3	122.1	139.11	-	-	-	-	-	-
8	194.3	200.7	101.6	101.7	101.4	101.50	-	-	-	-	-	-
9	129.6	129.9	125.7	125.8	125.5	125.64	5.81 (1H, s)	5,9 (1H, q, 1.5 Hz)	5.74 brs	5.76 (1H,s,H9)	5.75 (1H, brs)	5.77 (1H,s)
10	152.2	160.8	137.2	137.5	139.0	137.28	-	-	-	-	-	-
11	134.6	135.8	122.3	122.4	137.1	122.20	-	-	-	-	-	-
12	22.2	21.9	22.4	22.5	19.0	22.30	1.62 (3H, s)	1,82 (3H, br s)	1.54 s	1.80 (3H,s,H-12)	1.81 (3H,s)	1.60 (3H,s)
13	23.4	22.5	18.9	19.0	22.4	18.91	1.71 (3H, s)	1,86 (3H, s)	1.61 s	1.65 (3H,s,H-13)	1.59 (3H,s)	1,67 (3H,s)
14	24.8	24.6	11.9	12.0	21.1	11.91	1.01 (3H, d, 6 Hz)	1,04 (3H,d, 6.3 Hz)	1.01 d (6.4)	1.03 (3H,d,J=6.35,H-14)	1.02 (3H,d, 6.0 Hz)	1.03 (3H,d,6.25 Hz)
15	12.1	13.1	21.0	21.1	12.0	21.00	1.82 (3H, s)	1,97 (3H, br s)	1.79 s	1.59 (3H,s,H-15)	1.66 (3H,s)	1.82 (3H,d)

The EF.a2 isolate had better DPPH antioxidant activity than EF.a1. Furthermore, the antioxidant activity of the EF.a2 isolate was also tested using the FRAP and ABTS methods (Figure 2). The ABTS test measures the relative antioxidant activity to scavenge ABTS as oxidant. The FRAP method is based on the capacity of antioxidants to reduce ferri-tripyridyltriazine to complex ferro-tripyridyl-triazine (Fe(II) TPTZ). This ability allows the methods to determine the amount of complex ferro-tripyridyl-triazine.

The purity of EF.a2 as a bioactive antioxidant compound was tested for purity by TLC using 3 types of mobile phases with different polarities and HPLC instruments. The purity test was conducted by TLC using 3 mobile phases, namely chloroform: acetone (1:1), chloroform: dichlormethane (6:1), and dichlormethane: chloroform (9:1). All the three profiles showed a single spot which was visualized by cerium sulfate with hRf 89, 43, and 16, respectively. The identification results of the EF.a2 isolate compound showed a white crystalline form which was highly soluble in chloroform: methanol (1:1). The melting range of the EF.a2 isolate was 96.6-96.8°C. The HPLC and gas chromatography (GC) of the EF.a2 isolate showed one peak with a retention time of 12,781 and an area of 350.338 (purity 100%)(Supplementary Figure 1).

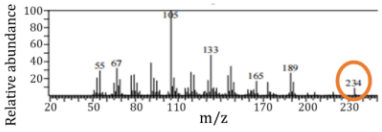

Figure 4. Molecular mass of EF.a2 isolated compound from *Curcuma aeruginosa* Roxb Rhizome

Based on the analysis by mass spectrometry, the EF.a2 compound had a molecular weight of 234 (M+) and base peak 105. Meanwhile, compound fragmentation which appeared at m/z peaks 189(37), 165(20), 143(20), 133(53), 121(29), 119(15), 105(100), 91(37), 67(37), 55(30) (Figure 4). The results of the identification of the EF.a2 identification were adjusted to the fragmentation patterns of curcumenol compounds in other studies which obtained the same results (Mukherjee *et al.*, 2011; Rajurkar & Hande, 2011; Setiawan *et al.*, 2021). At the FTIR (ATR) peak, the relevant bonds were determined as –C=C stretching (1456 cm^{-1}), -C=O stretching (1653 cm^{-1}), –C–H stretching (2956 cm^{-1}), =C–H stretching (3314 cm^{-1}), and O–H stretching (3364 cm^{-1}). On the UV Vis spectrophotometer (MeOH), there was a strong peak at 260 nm (Supplementary Figure 2). The results of the FTIR identification (Supplementary Figure 2) of the EF.a2 isolate were the same as the results of the curcumenol spectra in other studies. Curcumenol had an IR ($CHCl_3$) spectrum νmax cm-1: 3432, 2934, 1457 and a UV (MeOH) spectrum λmax nm (log ε): 248 (3.92)(Hamdi *et al.*, 2015). Signals of of ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (100MHz, $CDCl_3$) were compared with those of the curcumenol compound signals. The signal similarities in the ^{1}H-NMR (Table I) (400 MHz, $CDCl_3$) and ^{13}C-NMR (100MHz, $CDCl_3$) spectra between the EF.a2 isolates and curcumenol (Mukherjee *et al.*, 2011; Nurcholis *et al.*, 2016; Rajurkar & Hande, 2011; Setiawan *et al.*, 2021). The ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum had signals at 1.006-5.812 ppm; the ^{13}C-NMR (100 MHz, $CDCl_3$) spectrum contained 15 carbon atoms. All the NMR spectra of the EF.a2 (Supplementary Figure 3) isolated from this spectral interpretation, the isolated compound (EF.a2) had differences in the C8 position. In other publication that discusses the ^{13}C-NMR (100 MHz, $CDCl_3$) spectra of curcumenol, there was a chemical shift in 101 ppm whereas no chemical shift was found in the EF.a2 isolate at that position. The C8 position of the isolate on the spectra ^{13}C-NMR (100 MHz, $CDCl_3$) spectra was more suitable with curcumenotone compounds (Table I).

In the ^{13}C-NMR (100 MHz, $CDCl_3$) spectra of the isolate, there was a chemical shift in approximately 200 ppm which characterizes the presence of ketones. This means that curcumenol was found in the form of the keto tautomerism, namely curcumenotone. Compounds in the keto tautomerism form can be more stable than their enol tautomerism form. This is similar to research on curcumin which also had the keto-enol tautomerism (Istyastono *et al.*, 2003). In several previous tautomerization studies, it was reported that curcumin tends to be found in the enol tautomerism form. However, alkyl substituted curcumin at C-4 was reported to decrease the stability of the enol tautomerism. The 4-isopropylcurcumin which is found in the keto tautomeric form. This process supposedly explains the effect of the C-4 substitution of curcumin on the antioxidant activity of curcumin and its derivatives (Istyastono *et al.*, 2003). Tautomerization can be defined as the rapid interconversion of both keto and enol forms. The enthalpy of formation (ΔHf) of

the keto tautomerism form is lower than ΔHf of the enol tautomerism form. This shows that curcuminoid derivatives are more stable when functioning as keto tautomerism than enol tautomerism (Saputra & Kalalinggi, 2022). Tautomerization and biological activity of curcumenol was found in only 1 research article according to Zhang (Zhang *et al*., 2019). On the ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum, there was no significant difference in the chemical shifts between the spectra of curcumenol and curcumenotone in the EF.a2 isolate, it can be said that both were similar. This is because the location of the H in the two compound structures was the same except for the displacement of the OH group which was originally at the C8 position to C5. At the C8 position the hydroxyl groups (-OH) of curcumenotone was replaced with carbonyl groups (-C=O). The APT-NMR (100 MHz, $CDCl_3$) spectrum showed 8 downward peaks which were secondary and quaternary carbons. In the spectrum of curcumenotone, there were 3 -CH2 groups and 5 quaternary carbon groups, namely carbons that do not directly bond with hydrogen. Based on the identification of NMR spectra ^{1}H-NMR (400 MHz. $CDCl_3$), ^{13}C-NMR (100 MHz, $CDCl_3$), and APT NMR (100 MHz, $CDCl_3$), suggested that curcumenotone is a tautomer of curcumenol and this was the case of the EF.a2 isolate (Figure 5A and 5B).

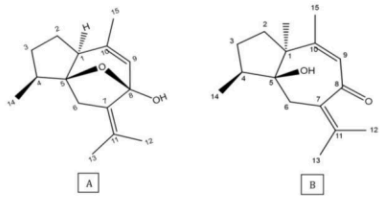

Figure 5. Structure of curcumenol (A) and curcumenotone (B)

CONCLUSION

EF.a2 has been isolated from *C. aeruginosa* Roxb rhizome by bioassay-guided isolation. The structure of the EF.a2 compounds was confirmed by modern spectrometry techniques. The identification result of Ef.a2 is a sesquiterpene compound $C_{15}H_{22}O_2$, namely curcumenotone, as a tautomer of curcumenol. The curcumenotone Isolate from the rhizome of *C. aeruginosa* has free radicals (DPPH and ABTS) scavenging and has reducing power (FRAP).

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