Evaluation of Free Radical Scavenging Activity of Ethanolic Extract from Promising Accessions of *Curcuma aeruginosa* RoxB.

Waras Nurcholis[1,2*], Nurul Khumaida[3], Muhamad Syukur[3], Maria Bintang[1]

[1]Department of Biochemistry, Bogor Agricultural University, Bogor, Indonesia
[2]Tropical Biopharmaca Research Center (Trop-BRC), Bogor Agricultural University, Bogor, Indonesia
[3]Department of Agronomy and Horticulture, Bogor Agricultural University, Bogor, Indonesia

*email: wnurcholis@apps.ipb.ac.id

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ABSTRACT

This study evaluated the antioxidant activity through the determination of free radical scavenging activity of ethanolic extracts from 20 accessions of *Curcuma aeruginosa*, and it is to use for the development of varieties in future. The radical scavenging activity of the extract accessions was investigated with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. IC_{50} values for DPPH radical scavenging activity ranged from 89.81 to 505.65 µg mL^{-1}. Based on IC_{50} values, twenty accessions of *C. aeruginosa* can be divided into three groups: strong (two accessions); moderate (seventeen accessions); and low (one accession) of DPPH scavenger. Sukoharjo (SH) and Muara Bungo (MB) showed promising accessions for antioxidant potential, thus these accessions important to selection for the future breeding program in pharmaceutical products.

Keywords*:* Antioxidant; breeding; DPPH; free radical; temu ireng;

INTRODUCTION

A free radical is defined as an atom or molecule containing one or more unpaired electron (Bayr, 2005) such as superoxide (O_2^-), hydroxyl (OH^-), peroxyl (RO_2^-) and hydroperoxyl (HO_2^-) (Halliwell & Gutteridge, 2015). Free radicals are the products from biochemical of normal cellular metabolism (Phaniendra, Jestadi, & Periyasamy, 2015). In vivo, the free radicals have a very reactive with lipid, protein, and DNA (Bhattacharya, 2015) to create a stable compound. Thus, excess of free radicals can seriously damage essential macromolecules of the cell and theirs implicated in many major diseases include cancer (Hecht et al., 2016), cardiovascular diseases (Tostes, Carneiro, Carvalho, & Reckelhoff, 2016), inflammatory diseases (Rimessi, Previati, Nigro, Wieckowski, & Pinton, 2016), heart diseases (Sverdlov et al., 2016), respiratory diseases (Guo et al., 2017), diabetes (Onoue et al., 2016), neurological diseases (Radak et al., 2016), aging process (Park, Sim, Lee, Sung, & Oh, 2016), etc. However, the human body has various mechanisms to counteract free radical by producing antioxidant: (a) enzymatic antioxidant i.e. superoxide dismutase, catalase, and glutathione peroxidase (Park et al., 2016); and (b) non-enzymatic antioxidants i.e. glutathione and nicotinamide adenosine dinucleotide phosphate (Kilanczyk, Saraswat Ohri, Whittemore, & Hetman, 2016). But, when the free radical production rate exceeds the capacity there will be a failure of the antioxidant defense system. Therefore, antioxidants need to be supplemented from outside sources.

During the last years, there has been interesting in scientific studies concerning discovery metabolite from the medicinal plant that can be applied to antioxidant (Jeong, Tulasi, & Koyyalamudi, 2016; Perera, Samarasekera, Handunnetti, & Weerasena, 2016). *Curcuma aeruginosa* RoxB. is a medicinal plant that contains phytochemical phenolic, flavonoid (Waras Nurcholis, Khumaida, Syukur, & Bintang, 2016b) and curcuminoids (Bos et al., 2007; Waras Nurcholis, Khumaida, Syukur, & Bintang, 2016a), so it's a potential source of natural antioxidant (Amalraj, Pius, Gopi, & Gopi, 2017; W Nurcholis, Khumaida, Syukur, Bintang, & Ardyani, 2015). *C. aeruginosa* has a broad geographical scope in Indonesia, which necessitates extensive investigation on the antioxidant attributes of their different accessions. Therefore, this study, we investigated the free radical scavenging activity of ethanol extract of 20 °*C. aeruginosa* promising accessions.

EXPERIMENTAL SECTION

C. aeruginosa rhizomes were collected in the month of February 2015 from different regions

of Indonesia (**Table 1**). Most of the accessions were pooled and taken for identified by experts at Tropical Biopharmaca Research Center, Bogor Agricultural University (IPB).

Preparation of ethanolic extract

The rhizome materials were sun dried to moisture content < 10% and then powdered (100 mesh). The resulting powder was subjected to extraction with 70% ethanol (W Nurcholis et al., 2015). The ethanolic extract was concentrated, using a rotary evaporator (BUCHI, R-250, Switzerland) and stored at 4 °C until used.

Determination of free radical scavenging activity

The stable 2,2-diphenyl-1-picrylhy drazyl (DPPH) analysis was used to determine free radical scavenging activity of the ethanolic extracts (25, 50, 100, 200, 400 and 800 µg mL^{-1}) (W. J. Li et al., 2012). Ethanolic extract (200 µL) in DMSO (Merck, Germany) was added to 50 µL of a methanol solution of DPPH. Absorbance at 517 nm was determined using microplate readers or microplate photometers (Epoch Biotech, USA) after incubation in dark for 30 min at 37 °C, and the percentage inhibition activity was calculated from $\left[\frac{A_0-A}{A_0}\right] x100$, where A_0 is the absorbance of the control, and A is the absorbance of the ethanolic extract. The inhibition curves were prepared and, IC_{50} values were obtained.

Statistical analysis

The data were analyzed using one-way variance analysis. The comparisons among the difference of means were further analyzed using Duncan's multiple range test at $p<0.05$. Analyses were performed using SPSS 19 statistical computer software.

RESULTS AND DISCUSSION

Analysis of the free radical scavenging activity of the ethanolic extract from 20 *C. aeruginosa* accessions tested by a stable free radical, namely DPPH as a reagent. The DPPH method is a preferred method because it is a convenient and fast technique to evaluate free radical scavenging or antioxidant activity (J. Li et al., 2013).

Table 1. Geographical collection sites of 20 *C. aeruginosa* accessions

Region accessions	Province	Latitude (S)	Longitude (E)	Altitude (m)
Madura (MD)	East Java	7˚02'48.90"	112˚43'47.32"	4
Kediri (KD)	East Java	7˚50'39.52"	111˚53'54.93"	489
Ponorogo (PR)	East Java	7˚51'51.47"	111˚28'11.78"	106
Pacitan (PT)	East Java	8˚11'59.56"	111˚06'13.34"	7
Ngawi (NW)	East Java	7˚29'52.21"	111˚09'22.78"	345
Karanganyar (KA)	Central Java	7˚39'49.37"	111˚08'01.93"	1113
Sragen (SG)	Central Java	7˚24'22.14"	111˚07'12.84"	90
Solo (GD)	Central Java	7˚34'08.83"	110˚49'54.53"	95
Solo (KL)	Central Java	7˚35'05.66"	110˚49'45.38"	96
Sukoharjo (SH)	Central Java	7˚44'41.62"	110˚52'41.14"	111
Wonogiri (WG)	Central Java	7˚57'22.83"	110˚59'37.51"	378
Purworejo (PW)	Central Java	7˚44'25.35"	110˚01'59.00"	56
Kendal (KN)	Central Java	7˚00'55.14"	110˚16'05.98"	78
Pakem (PK)	Yogyakarta	7˚39'55.46"	110˚25'11.30"	424
Beringharjo (BH)	Yogyakarta	7˚47'56.40"	110˚22'01.56"	115
Kulonprogo (KP)	Yogyakarta	7˚56'25.03"	110˚14'20.30"	20
Gunung Kidul (GK)	Yogyakarta	7˚58'04.87"	110˚36'09.67"	180
Cirebon (LC)	West Java	6˚48'17.09"	108˚48'06.04"	1
Bogor (CB)	West Java	6˚32'35.89"	106˚41'22.41"	148
Muara Bungo (MB)	Jambi	1˚37'00.61"	102˚22'16.28"	65

The DPPH radical is a deep purplish color, which is at its maximum wavelength at 515-520 nm and can change color from violet to yellow if received an electron or hydrogen from antioxidant molecules to become a stable DPPH molecule (Carmona-Jiménez, García-Moreno, Igartuburu, & Barroso, 2014; Pérez & Aguilar, 2013). Therefore, the discoloration of the DPPH radical reflects the free radical scavenging activity of the analyzed extract in 20 *C. aeruginosa* accessions (**Figure 1**). The percentage inhibitions of DPPH assay from the ethanolic extract of twenty *C. aeruginosa* promoting accession are given in **Figure 2**, and the IC_{50} values are presented in **Figure 3**. The DPPH radical scavenging capacity was evaluated regarding percent reduction of the initial DPPH absorption. The percentages of inhibition of the Sukoharjo (SH) and Muara Bungo (MB) accessions demonstrated superior DPPH radicals scavenging activity than the others accessions at 25-800 µg mL^{-1}.

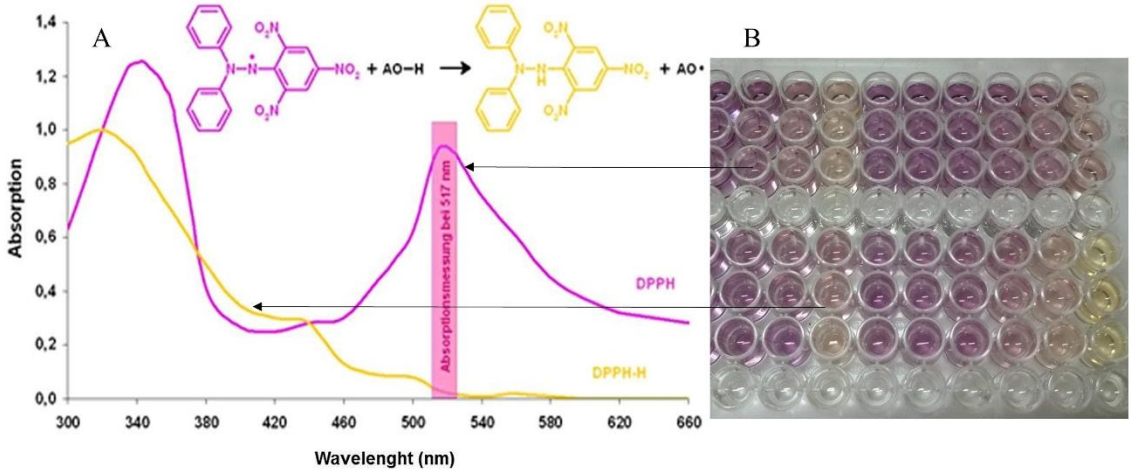

Figure 1. The color change of DPPH: (A) from purple to yellow in graph (Pérez & Aguilar, 2013) and sample of *C. aeruginosa* in 96-micro plate well (B), when it exposed to antioxidant substance

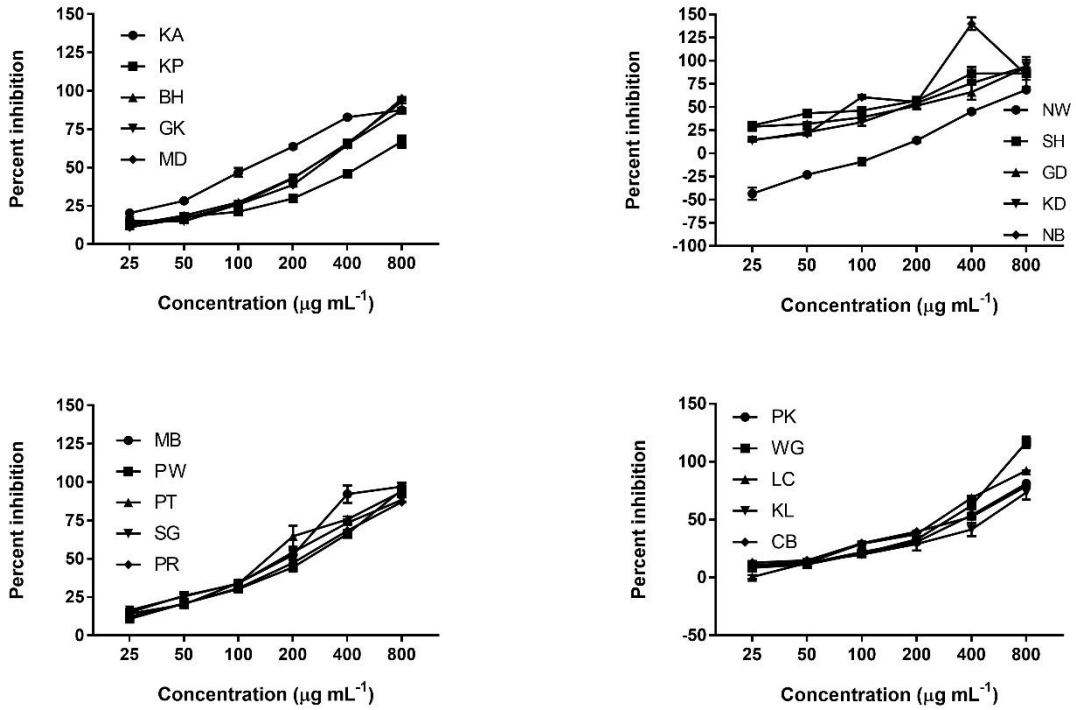

Figure 2. Inhibition activity of twenty *C. aeruginosa* promising accessions ethanolic extracted from DPPH radicals

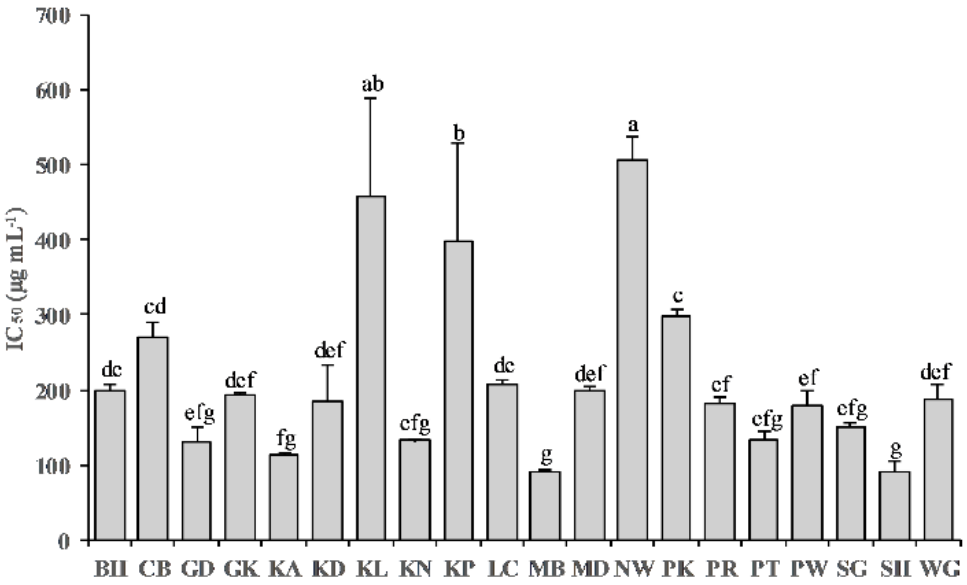

Figure 3. Free radical-scavenging activity in an ethanolic extract of twenty *C. aeruginosa* promising accessions by DPPH assay. Each data represents the mean ± SD of three replicates. Values with different lowercase letters represent significant differences at $p<0.05$.

The free radical scavenging reported as IC_{50} values that defined as the concentration of sample extract necessary to obtain an activity of 50% of the DPPH radicals (Nickavar, Alinaghi, & Kamalinejad, 2008). The lower IC_{50} value indicates higher free radical scavenging activity. The IC_{50} values in the ethanolic extract of *C. aeruginosa* accession ranged from 89.91 to 505.65 µg mL^{-1}. Accessions of Sukoharjo (SH) and Muara Bungo (MB) had a significantly higher free radical scavenging ($p<0.05$) compared to others accessions except with accessions of Solo (GD), Kendal (KN), Pacitan (PT) and Sragen (SG). According to IC_{50} values, the free radical scavenging activity can be divided into three groups: (a) strong with $IC_{50}<100$ µg mL^{-1}; (b) intermediate with IC_{50}, 100-500 µg mL^{-1}; and (c) weak with $IC_{50}>500$ µg mL^{-1} (Bi et al., 2016). Therefore, 20 accessions of *C. aeruginosa* can be divided three groups based on IC_{50} values. The first group, which is comprised accessions of Sukoharjo (SH) and Muara Bungo (MB), was defined by strong of the radical scavenging activity. The second group consisted of seventeen accessions, including Ponorogo (PR), Pacitan (PT), Karanganyar (KA), Sragen (SG), Solo (GD), Solo (KL), Wonogiri (WG), Purworejo (PW), Kendal (KN), Pakem (PK), Beringharjo (BH), Kulonprogo (KP), Gunung Kidul (GK), Cirebon (LC), Bogor (CB), Madura (MD), and Kediri (KD): this group has relatively intermediate of the free radical scavenging activity. The third group included one accessions of Ngawi (NW) which exhibited relatively low of the radical scavenging activity.

In literature, there are some reports regarding free radical scavenging of *C. aeruginosa* tested by the DPPH method. Based on our result, free radical scavenging capacity in ethanolic extract of twenty *C. aeruginosa* accessions are lower than essential oil extract with IC_{50} values of 28 µg mL^{-1} (George & Britto, 2015) and 24 28 µg mL^{-1} (Theanphong, Mingvanish, & Kirdmanee, 2013). In another report, the free radical scavenging of the ethanol and oleoresins extracts were 437.07 µg mL^{-1} (W Nurcholis et al., 2015) and 450 µg mL^{-1} (Rajamma, Bai, & Nambisan, 2012), respectively. The ethanolic extract accessions of Sukoharjo (SH) and Muara Bungo (MB) were shown strong the free radical scavenging activity with IC_{50} values of 89.81 and 90.87, respectively. Accessions of SH and MB had strong to potent antioxidant activity. This result provides important information for selecting high-quality *C. aeruginosa* rhizome in the future breeding program for antioxidant activity.

CONCLUSION

Among all the accession of *C. aeruginosa*, Sukoharjo (SH) and Muara Bungo (MB) showed promising accessions for antioxidant

potential by free radical scavenging activity. Thus, these accessions important to selection for the future breeding program in pharmaceutical products.

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