Antioxidant and antibacterial activities of rhizomes extracts

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RESEARCH ARTICLE

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Abstract *Curcuma aeruginosa Curcuma zedoaria*, recognized as Temu Hitam and Temu Putih, is widely used as traditional herbal in Indonesia. Many biological activities and secondary metabolites of *C. aeruginosa C. zedoaria* had been reported. This research was aimed at the antioxidant and antibacterial activities of *C. aeruginosa C. zedoaria* rhizomes extracts using ethanol activities by using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method. The ethanol extract of *C. aeruginosa* presented antioxidant activity with IC_{50} of 0,67 ppm compared to gallic acid as a standard. Meanwhile, the antibacterial activity has been determined by using colorimetric resazurin microtiter assay (REMA) method. The *C. zedoaria* extract showed the highest antibacterial activity against *B. subtilis*, *S. aureus, P. acnes*, *P. aeruginosa* and *S. typhi* with MIC of mg/mL, respectively compared to ampicillin as a standard. Furthermore, the extracts have been assayed their antibacterial activities against both gram-positive and negative bacteria. This research showed that *Curcuma aeruginosa and Curcuma zedoaria* rhizomes extracts have potency as antioxidant.

Keywords: Antibacterial, Antioxidant, *Curcuma aeruginosa*, *Curcuma zedoaria*, DPPH, REMA.

Introduction

Free radicals are compounds that have one or more unpaired electrons. Free radicals can be generated from the body's metabolism and radiation, UV rays and cigarette smoke. Free radical attack cause cancer, cataracts, decreased kidney function, and atherosclerosis or narrowing of blood vessels which. Free radicals can also cause cell damage that make person. Antioxidants prevent the adverse effects of free radicals. Antioxidants are compounds which can inhibit reactive oxygen species/nitrogen species reactive (ROS/RNS) and free radicals [1].

A massive number of human, animal, and plant sicknesses are pathogenic microbes together with fungi, bacteria and algae. The contamination fungi and microorganism in higher organisms. Antibacterial agent is the compound used to control the of harmful bacteria [2]. Therefore, necessary to find a new source of antioxidant and antibacterial from natural. Indonesia has a huge biodiversity of medicinal herbs and species. One of the medicinal herbs is an herbaceous plant (*Zingiberaceae*). This herb is one of rhizome tropical plants that is very useful. The rhizomes of several plants are used as species, medicines, cosmetic ingredients and flavour. *C. aeruginosa* (Temu Hitam) and *C. zedoaria* (Temu Putih) are classified as curcuma species. Both of these plants are easy to cultivate and abundant in Indonesia. *C. aeruginosa* is one of the widely cultivated Curcuma species in tropical and subtropical regions such as Indonesia [3], India [4-5], Myanmar, Cambodia [6], Malaysia [7], Thailand [8], Bangladesh [9-10], Vietnam [11] and Japan [12]. *C. aeruginosa* is used as a traditional medicinal herb in the form of a mixture or in a single form. In Indonesia, people used these two plants as a traditional drink to treat stomach pain, diarrhea, and increase appetite. The plant consist of high amount essential oil and volatile constituents. It's also have a potent antioxidant activity and antimicrobial properties in pharmaceutical, food preservation and flavoring, and natural therapies [4,7]. The chemical compounds contained in the rhizome are curcumin, zedoarin, gum, resin, starch, saponin, flavonoid, polyphenol, and essential oils [13]. Medicinal plants have attracted many scholars because of their pharmacological properties [14].

A black turmeric (*C. aeruginosa*) can be used as a medicinal plant because extracts of black turmeric are reported as antibacterial and anti-inflammatory, antinociceptive, and antipyretic agents [15]. This essential oil of black turmeric has antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. Secondary metabolites of black turmeric are flavonoids, terpenoids, saponins, tannins, and alkaloids [16]. The bioactivities of secondary metabolites the growing place, climate, and conditions around the plant [17].

A white turmeric (*C. zedoaria*) is commonly used by people to relieve typhoid fever. The ethnobotanical approach was used as the basis of this research. The advantage of using both white and black turmeric as a natural antibacterial agent is safer than that of synthetic antioxidant and antibacterial. The use of synthetic food additives raises many concerns about adverse health effects. This study aimed to compare the antioxidant and antibacterial activities of white and black turmeric rhizome extracts and to determine the minimum inhibitory concentration (MIC) of white and black turmeric rhizomes against *Bacillus subtilis*, *Staphylococcus aureus*, *Propionibacterium acnes*, *Pseudomonas aeruginosa*, and *Salmonella typhi*.

Materials and Methods

Chemicals

Ethanol was used as an organic solvent for extraction. DPPH (Aldrich, 1898-66-4),methanol (Merck, 1.06009.2500) and gallic acid (Aldrich, 149-91-7) were used for antioxidant assay. DMSO (Merck, 1.02952.1000), Mueller Hinton Agar (MHA) (HIMEDIA, M173-500G), Mueller Hinton Broth (MHB) (HIMEDIA, M391-500G), aquadest, McFarland 0.5 (HIMEDIA, R092-1NO), resazurin (Aldrich, 199303-1G), ampicillin, and gram-positive and negative of isolate bacteria included *B. subtilis* (ATCC-19659), *S. aureus* (ATCC-29213), *S. typhimurium* (FNCC-0050), *P. aeruginosa* (ATCC-27853), and *P. acnes* (ATCC-6919) were used as materials for antibacterial assay system.

Plant materials, sample preparation and extraction

The rhizomes of *C. aeruginosa* and *C. zedoaria* were collected from Jombang, East Java, Indonesia (7° 33' 0" S, 112° 14' 0" E) on December 2021. The *Curcuma aeruginosa* and *Curcuma zedoaria* rhizomes were dried for 20 days at room temperature. The dried rhizomes were extracted with ethanol by using maceration at room temperature.

In vitro DPPH radical scavenging assay

Antioxidant activity of *C. aeruginosa* and *C. zedoaria* extracts was conducted by in vitro DPPH radical scavenging assay based on the previous reported research with minor modification [18]. Stock extract solution was prepared by using methanol with concentration of 10 mg/mL. A working solution of DPPH was dissolved in methanol with concentration of $6x10^{-5}$ M. Furthermore, the test solution was conducted by adding extract and DPPH solution with volume of 49.5 μL and 1.5 mL, respectively. The absorbance value of the mixed test solution or called as sample absorbance (As) was determined by using UV-Vis spectrophotometer with wavelength of 517 nm. The blank absorbance (Ab) was measured with DPPH solution and methanol only. Gallic acid was used as a standard or positive control. The inhibitory activity was calculated by using equation (1). IC_{50} value was determined by analysis of either regression equation linear or polynomial.

$$\text{Inhibitory activity (\%)} = [(Ab-As)/Ab] \times 100\% \qquad (1)$$

Antibacterial assay

Antibacterial assay was conducted by microdilution method namely REMA with resazurin indicator [19]. REMA is based on an ability of cell to reduce a resazurin compound with blue colour to be a resorufin compound in pink colour biologically. The best tool of microdilution method is 96-well plates to save amount of extract. Furthermore, antibacterial assay of *C. aeruginosa* and *C. zedoaria* extracts was done by the following:

Extract preparation

Extracts of ethanol were prepared on DMSO with concentration of 100 mg/mL. This stock extract solution was dissolved by using aquadest at concentration of 20 mg/mL. This concentration was used as the highest concentration on antibacterial activity assay.

Medium preparation

In this assay system, the bacterial medium was MHA for re-culture bacterial and MHB for the tested bacterial suspension. MHA was prepared of 9.5 g on aquadest of 250 L. Meanwhile, MHB was prepared. In addition, MHB at concentration of 3.3 x was also prepared. All prepared medium was sterilized by using autoclave at temperature of 121°C for 15 minutes.

Bacterial culture preparation

The antibacterial assay was used isolated bacteria gram-positive and negative. The isolated bacteria are *B. subtilis*, *S. aureus*, *S. typhimurium*, *P. aeruginosa*, and *P. acnes*. The tested bacteria were cultured on MHA medium and incubated for 1x24 hours at temperature of 37°C. Before antibacterial assay with REMA method was started, the bacterial suspension in MHB was prepared. The bacterial suspension was made by using the cultured bacteria in MHA and compared to a standard of McFarland 0.5. This compared bacterial suspension aims to get the tested bacteria at concentration of $5x10^6$ cfu/mL.

Resazurin reagent preparation

Resazurin stock solution was prepared on aquadest at concentration of 0.01 g/mL. Before REMA was been started, the stock solution was diluted to be a working solution with ratio of 1:10.

Colorimetric Resazurin Microtiter Assay (REMA)

As mentioned before, REMA with resazurin as an indicator was used to this antibacterial assay system. First, the prepared extract was added to first holes of 96-well plates with volume of 50 μL at concentration of 20 mg/mL. The extract solution was added with twice dilution by using sterile aquadest until the 8^{th} of hole. After that, the prepared resazurin solution and MHB were added to the well hole with volume of 10 μL and 30 μL, respectively. The bacterial suspension ($5x10^6$ cfu/mL) with volume of 10 μL was added on the well hole. The well plates were closed and incubated for 24 hours at temperature of 37°C.

Antibacterial activity on the well plates could be identified because of indicator discoloration. If there is the indicator discoloration from blue to pink, it means the bacteria are still growing. In contrast, if there is no indicator discoloration, it means the extract could inhibit the bacteria activity. Minimum inhibitory concentration (MIC) value has been evaluated by the lowest concentration of the tested extract. A negative control was prepared with no extract. Ampicillin was used as a positive control.

Results and Discussion

Extraction

The extracts of *C. aeruginosa* and *C. zedoaria* rhizomes from ethanol solvents have been obtained. The ethanol extract of *C. aeruginosa* rhizomes has the highest yield of 5,67%. The ethanol extract of *C. zedoaria* rhizomes yields of 3,25%.

Antioxidant activity

The antioxidant activity results among the various extracts are shown in Fig. 1. The value of inhibition (%) of extracts based on DPPH assay at a concentration of 319.46 μg/mL were obtained. According to the data, the ethanol extract of *C. aeruginosa* rhizomes showed the highest inhibition about 80.34%. Gallic acid (positive control) showed antioxidant activity about 91.8%.

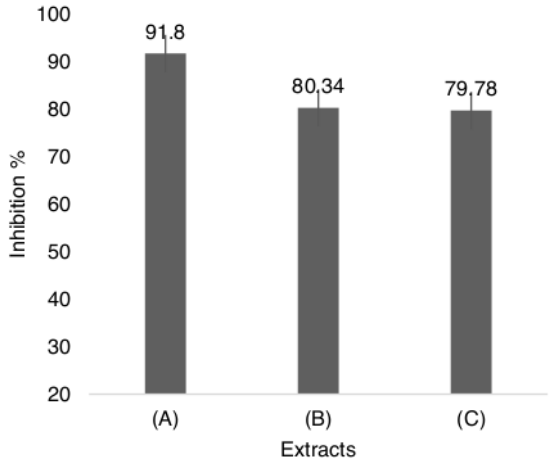

Figure 2. DPPH radical scavenging activity screening of ; (A) gallic acid, (B) the ethanol extract of *C. aeruginosa* rhizome (C) the ethanol extract of *C. zedoaria* rhizomes. Each bar represents the mean ± SD, n=3 at concentration of 319.46 µg/mL.

The ethanol extracts of *C. aeruginosa* rhizomes and *C. zedoaria* rhizomes were measured for their IC_{50} value (**Figure 2.**). Gallic acid was used as positive control.

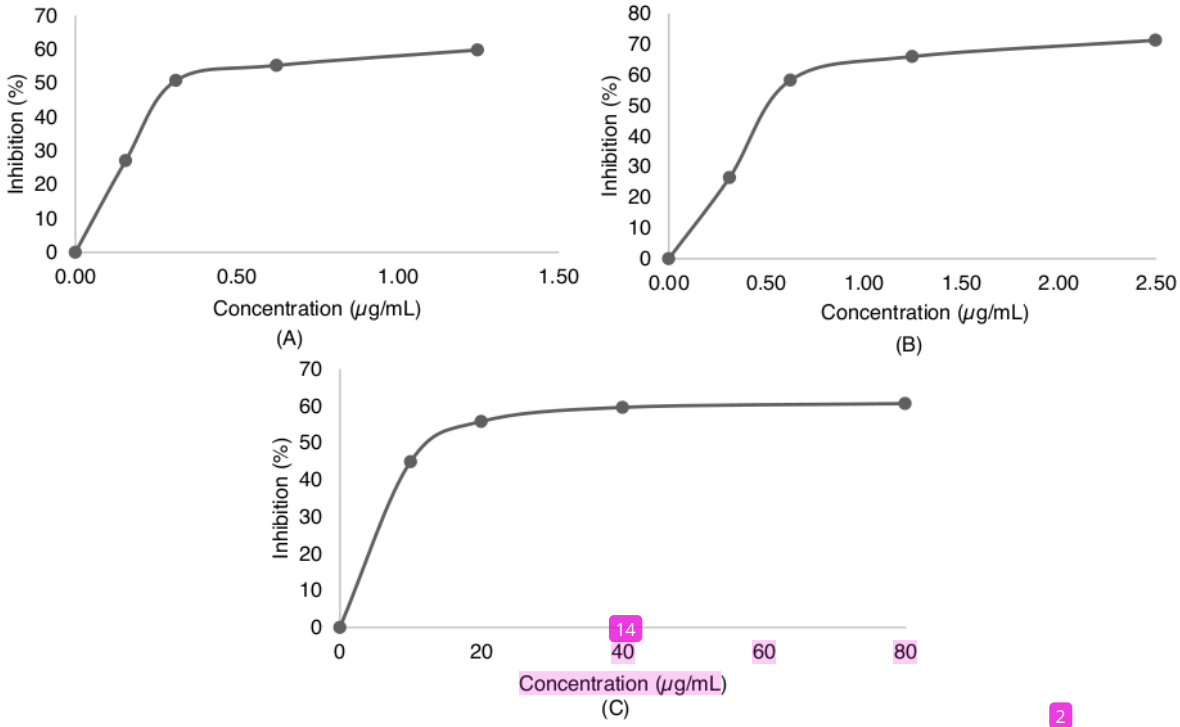

Figure 2: DPPH scavenging activity of; (A) gallic acid, (B) the ethanol extract of *C. aeruginosa* rhizomes, (C) the ethanol extract of *C. zedoaria* rhizomes. Each bar represents the mean ± SD, n=3 at concentration of 319.46 µg/mL.

The ethanol extract of *C. aeruginosa* rhizomes has IC_{50} value about 0.67 µg/mL as shown in Fig. 2. While the value of IC_{50} the ethanol extract of *C. zedoaria* rhizomes was 15.11 µg/mL. The lowest of IC_{50} value indicate a good free radical scavenging activity [20]. This indicated that the ethanol extract of *C. aeruginosa* rhizomes has the higher antioxidant activity than that of the ethanol extract of *C. zedoaria* rhizomes. This result could play a valuable role in pharmaceutical and food conservation to prevention of oxidative damage related to the pathophysiology of several diseases. The same result had been reported that the essential oil of the rhizome of *C. aeruginosa* showed a good antioxidant activity [10].

Antibacterial activity

Five different bacteria strains were used to study the antibacterial activity of the ethanol extracts of *C. aeruginosa* rhizomes and *C. zedoaria* rhizomes. The selected bacteria include three Gram-positive (*B. subtilis and S. aureus*) and two Gram-negative (*S. typhi, P. acnes* and *P. aeruginosa*) bacteria. The results are shown in Table 1.

Table 1. Antibacterial activities of ethanol extracts of *C. aeruginosa* rhizomes and *C. zedoaria* rhizomes

No	Extracts	MIC* (mg/mL)				
		B. subtilis	*S. aureus*	*S. typhimurium*	*P. aeruginosa*	*P. acnes*
1	ethanol extracts of *C. aeruginosa*	0.63	>10	10	>10	1.25
2	ethanol extracts of *C. zedoaria*	0.63	5	1.25	10	0.31
3	Ampicilin	0.31	<0.08	2.50	5.00	0.63

The ethanol extract of *C. aeruginosa* showed a good inhibition activity against *B. subtilis* and *P. acnes*. Whereas the ethanol extracts of *C. zedoaria* are potent against *P. acnes* and *S. typhimurium*. The ethanol extracts of *C. zedoaria* have the highest activity against *S. typhimurium* with MIC value about 1.25 mg/mL. *C. zedoaria* can be used to assist in the healing of typhoid disease caused by the bacterium *S. typhimurium*. The same result had been reported the preliminary antibacterial assay of methanol extract of C. aeruginosa rhizomes on *S. typhi* and *Escherichia* coli showed moderate activity [14]. It can be suggested that curcumenol played an important contribution to an antibacterial activity toward Gram-negative bacteria. Based on those results, *C. aeruginosa* rhizome was proposed to have excellent antibacterial activity for therapeutic purposes. However, further studies need to characterize the bioactive antibacterial compound and understand its molecular mechanism of action.

Conclusions

The extracts of *C. aeruginosa* rhizhomes showed a high antioxidant activities using DPPH methods with IC_{50} 0.67 µg/mL. The ethanol extracts of *C. zedoaria* have the highest activity against *S. typhimurium* with an MIC value about 1.25 mg/mL. The antibacterial activity showed that these extracts can be used to assist in the healing of typhoid disease caused by the bacterium *S. typhimurium*. This study indicated that *C. aeruginosa* and *C. zedoaria* rhizhomes could be used as a antioxidant and antibacterial agent.

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