

ANTIBACTERIAL ACTIVITY OF CURCUMENOL FROM RHIZOMES OF INDONESIAN *CURCUMA AERUGINOSA* (ZINGIBERACEAE)

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ABSTRACT

The rhizomes of *Curcuma aeruginosa* (Zingiberaceae), locally known as *Temu Hitam*, is usually used as a traditional medicine. The bioactive compounds in this plant were known to have antibacterial activities. However, information regarding bioactive compounds on antibacterial activity contained in *C. aeruginosa* rhizomes is still limited. In continuing our study on Indonesian medicinal plants, the isolation of bioactive compounds from *C. aeruginosa* growing in Indonesia had been conducted. Curcumenol had been isolated from the methanol extract of *C. aeruginosa* rhizomes by using extraction methods and several chromatography techniques, i.e. vacuum liquid, radial, and preparative thin layer chromatography. Furthermore, this compound had been elucidated based on one-dimensional NMR (¹H and ¹³C) and MS. The preliminary antibacterial assay of methanol extract of *C. aeruginosa* rhizomes on *Salmonella typhi* and *Escherichia coli* showed moderate activity with an inhibition zone of 7 mm (inhibition index of 1.17) and 6 mm (inhibition index of 1.00), in 50 ppm, respectively. Moreover, curcumenol also exhibited moderate activity in 50 ppm with 8 mm of inhibition zone (1.33 of inhibition index) on *S. typhi* while on *E. coli* showed weak activity in 50 ppm with 4 mm of inhibition zone (0.67 of inhibition index). However, both the methanol extract of *C. aeruginosa* rhizomes and curcumenol were inactive on *Bacillus cereus* and *Staphylococcus aureus*. It can be suggested that curcumenol played an important contribution to an antibacterial activity toward Gram-negative bacteria (*S. typhi* and *E. coli*) in *C. aeruginosa* rhizomes.

Keywords: antibacterial, *Bacillus cereus*, *Curcuma aeruginosa*, *Escherichia coli*, curcumenol, *Salmonella typhi*, *Staphylococcus aureus*.

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INTRODUCTION

Indonesia has the highest biodiversity in the world reaching 11% of plants species found in the Earth's surface. Eighty percent of them are known as medicinal plants, but only around 1,000 species which have been used as traditional medicines.¹ One of the species of *Curcuma* genus (Zingiberaceae), *C. aeruginosa*, locally known as *Temu Hitam*, is usually used in traditional medicines for treating various ailments.² The previous researches reported that the variety of compounds in this plants extract, such as phenolic compounds, flavones, lignans, and terpenes derivatives, have also been known to have antibacterial and anticancer activities.³⁻⁹ However, there are no recent studies that have been reported regarding bioactive compounds on antibacterial activity contained in *C. aeruginosa* rhizomes. Here, we focused on the isolation of bioactive compound on antibacterial activity towards Gram-negative bacteria (*S. typhi* and *E. coli*) and Gram-positive bacteria (*B. cereus* and *S. aureus*) and found that curcumenol is a moderate-active compound isolated from *C. aeruginosa* rhizomes.

EXPERIMENTAL

General Experimental Procedures

¹H NMR (500 MHz) and ¹³C NMR (125 MHz) spectra were recorded in CDCl₃ using Agilent 500 instrument. Chemical shift references were obtained by addition of TMS. MS spectra were measured using

GC/MS Agilent 19091S-433 instrument. Melting point was determined using Fisher-Johns melting point apparatus. Vacuum liquid chromatography was performed using Si 60 G (Merck) for column packed and Si 60 (0.2-0.5 mm) (Merck) for sample adsorbed. Radial chromatography was carried out using Si 60 PF₂₅₄ containing gypsum (Merck). Si 60 GF₂₅₄ (Merck) was used for preparative TLC. For TLC analysis, pre-coated silica gel plates (Merck Si 60 GF₂₅₄, 0.25 mm thickness) and Ce(SO₄)₂.4H₂O 1.5% in H₂SO₄ 2N as apparition stain reagent were used. Antibacterial activity was conducted using disc diffusion methods. Four bacteria, i.e. *S. typhi*, *E. coli*, *B. cereus*, and *S. aureus* from Departement of Biology IPB were used for antibacterial activity assays. Tetracycline was selected as positive control while DMSO was used as negative control. Inhibition index was measured by the following equation (Equation 1).

$$\text{Inhibition index} = \frac{\text{Inhibition zone of sample}}{\text{Paper disc diameter}} \quad (1)$$

Plant Materials

C. aeruginosa rhizomes were collected from Pusat Studi Biofarmaka Tropika (Trop BRC) LPPM-IPB, West Java, Indonesia in January 2017.

Extraction and Isolation

Dried powdered *C. aeruginosa* rhizomes (1.01 kg) were exhaustively extracted three times with MeOH at room temperature. After filtering and evaporating the solvent, 118.74 g crude extract was yielded. The crude extract (30 g) was then fractionated using vacuum liquid chromatography with *n*-hexane:EtOAc as a solvent to obtain seven major fractions (A-H). Fraction C (3.05 g) was separated by using repeated vacuum liquid chromatography with *n*-hexane:EtOAc as a solvent yielding seven sub-fractions (C1-C7). Curcumenol (6.0 mg; 0.59% yield) was isolated from sub-fraction C6 after separating and purifying by using radial chromatography with *n*-hexane and increasing polarity as a solvent then followed by using preparative TLC with *n*-hexane:CHCl₃ 3:7 as a solvent.

Curcumenol, C₁₅H₂₂O₂; white powder; melting point: 117-119.5°C; *R_f* value: 0.875 in *n*-hexane:CHCl₃ 3:7; ¹H NMR (CDCl₃): 5.76 (1H, *s*, H-9), 4.53 (1H, *br s*, -OH in C-8), 2.88 (1H, *s*, H-6a), 2.66 (1H, *d*, J = 15.8, H-6b), 2.00 (1H, *m*, H-1), 1.93 (4H, *m*, H-2, H-3), 1.89 (1H, *m*, H-4), 1.80 (3H, *s*, H-12), 1.65 (3H, *s*, H-13), 1.59 (3H, *s*, H-15), and 1.03 (3H, *d*, J = 6.35, H-14); ¹³C NMR (CDCl₃): 139.3 (C-7), 137.5 (C-10), 125.8 (C-9), 122.4 (C-11), 101.7 (C-8), 85.8 (C-5), 51.4 (C-1), 40.5 (C-4), 37.3 (C-6), 31.4 (C-3), 27.8 (C-2), 22.5 (C-12), 21.1 (C-15), 19.0 (C-13), and 12.0 (C-14); MS (*m/z*): 234.

RESULTS AND DISCUSSION

C. aeruginosa, belonging to Zingiberaceae family, was chosen for present study by an analysis of the published literature that showed that this species is usually used as a traditional medicine and its extract had antibacterial activity.^{2,5,7} However, there are no recent studies have been reported on antibacterial bioactive compounds from Indonesian *C. aeruginosa*.

Initially, dried powdered of *C. aeruginosa* rhizomes was extracted with MeOH. The MeOH extract was tested on antibacterial activity and demonstrated moderate activity toward *S. typhi* and *E. coli* with an inhibition zone of 7 and 6 mm, respectively, (Table 1) and inhibition index of 1.17 and 1.00, respectively, (Fig.-1), in 50 ppm. In contrast, this extract was inactive toward *B. cereus* and *S. aureus*. This antibacterial activity, especially toward Gram-negative bacteria (*S. typhi* and *E. coli*), enriched the information regarding *Curcuma* extract having antibacterial activities. Previous studies had been reported that besides *C. aeruginosa* extract, the others *Curcuma* extract also had antibacterial activity, such as *C. heyneana*, *C. zedoaria*, *C. longa*, and *C. xanthorrhiza*.^{5,7,10-14} This extract was then subjected to various chromatography techniques resulting in one known guaiane-type sesquiterpenes, curcumenol (Fig.-2).

The structure elucidation of curcumenol was carried out based on one-dimensional NMR (¹H and ¹³C) and also compared with previously reported data^{4,8,15}. Based on ¹³C NMR spectra, there were 15 signals of carbon which correspond to sesquiterpenes derivatives, i.e. 4 C-*sp*² from alkenes double bonds in δ_C of 122.4-139.3 ppm, 2 C-*sp*³ bonded to O given the de-shielding δ_C of 85.8-101.7 ppm, and 9 C-*sp*³ signals in

δ_C of 12.0-51.4 ppm. 1H NMR spectra showed that this compound had 22 signals which correspond to 1 H-C sp^2 alkenes in δ_H of 5.76 ppm (1H, s), 1 H bonded to hydroxyl group in δ_H of 4.53 ppm (1H, br s), 2 H-C sp^3 bonded to C sp^2 (electron withdrawing group) yielded the de-shielding δ_H of 2.66-2.88 ppm, and the rest were H-C sp^3 in δ_H of 1.03-2.00 ppm. The molecular formula concluded from NMR spectral data was C₁₅H₂₂O₂ with 5 degree of unsaturated, i.e. 2 alkenes double bonds and 3 cyclics. Therefore, it was no doubtful that the isolated compound was curcumenol. Curcumenol previously isolated from Malaysian *C. zedoaria* and also Thailand and Vietnam *C. aeruginosa*^{4,8,15}.

Table-1: Antibacterial activity of MeOH extract of *C. aeruginosa* rhizomes and curcumenol towards *S. typhi*, *E. coli*, *B. cereus*, and *S. aureus*.

Bacteria	Conc. (ppm)	Inhibition Zone (mm) ^{a,b}		Activity Classification ^{c,16}	
		MeOH extract	Curcumenol	MeOH extract	Curcumenol
<i>S. typhi</i> (Gram -)	3.12	4	4	Weak	Weak
	6.25	5	4	Moderate	Weak
	12.50	6	5	Moderate	Moderate
	25.00	6	6	Moderate	Moderate
	50.00	7	8	Moderate	Moderate
<i>E. coli</i> (Gram -)	3.12	5	5	Moderate	Moderate
	6.25	5.5	4	Moderate	Weak
	12.50	4	4	Weak	Weak
	25.00	5.5	3.5	Moderate	Weak
	50.00	6	4	Moderate	Weak
<i>B. cereus</i> and <i>S. aureus</i> (Gram +)	3.12	~0	~0	Inactive	Inactive
	6.25	~0	~0	Inactive	Inactive
	12.50	~0	~0	Inactive	Inactive
	25.00	~0	~0	Inactive	Inactive
	50.00	~0	~0	Inactive	Inactive

^a Inhibition zone was measured in compared with paper disk diameter (6 mm).

^b Inhibition zones of positive control (tetracycline) were 49, 44, 14, and 14 mm for *S. typhi*, *E. coli*, *B. cereus*, and *S. aureus*, respectively, in 100 ppm. Tetracycline classified as very strong activity toward *S. typhi* and *E. coli* and strong activity toward *B. cereus* and *S. aureus*.^{c,16} Negative control (DMSO) did not show any activity on four bacteria. All inhibition zones for positive control were also measured in compared with paper disk diameter (6 mm).

^c Activity classification based on W. W. Davis and T. R. Stout, 1971: weak (< 5 mm), moderate (5-10 mm), strong (10-20 mm), and very strong (> 20 mm)

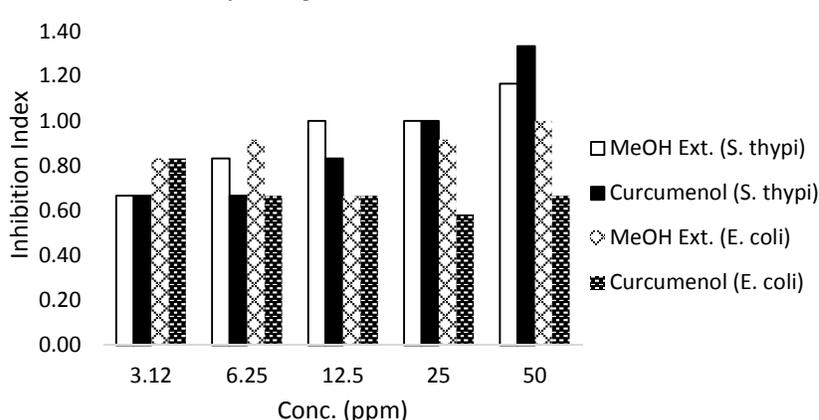


Fig-1: Inhibition index toward *S. typhi* and *E. coli* from MeOH extract and curcumenol

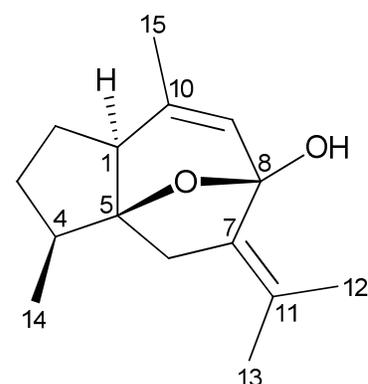


Fig-2: The structure of curcumenol

The ability to inhibit bacterial growth toward *S. typhi*, *E. coli*, *B. cereus*, and *S. aureus* of curcumenol using disc diffusion method had been examined. This assay is based on the formation of clear zone around the paper disc. This is the first report on antibacterial activity toward *S. typhi*, *E. coli*, *B. cereus*, and *S. aureus*

of curcumenol. Curcumenol exhibited moderate activity in 50 ppm with 8 mm of inhibition zone (Table-1) and 1.33 of inhibition index (Fig.-1) on *S. typhi* and showed weak activity towards *E. coli* with 4 mm of inhibition zone (Table-1) and 0.67 of inhibition index (Fig.-1). In contrast, this compound was inactive toward *B. cereus* and *S. aureus*.

Figure-1 showed that increasing concentration mostly will increase the inhibition index. Inhibition index of curcumenol towards *S. typhi* was higher than *E. coli*. Inhibition index of curcumenol in ≤ 25 ppm showed a similarity inhibition with MeOH extract, while in > 25 ppm showed higher inhibition than MeOH extract on *S. typhi*. It can be suggested that curcumenol played an important contribution to an antibacterial activity toward Gram-negative bacteria (*S. typhi* and *E. coli*) in *C. aeruginosa* rhizomes.

CONCLUSION

Curcumenol had been isolated from MeOH extract of Indonesian *C. aeruginosa* rhizomes. Formerly, this compound was obtained from Malaysian *C. zedoaria* and Thailand and Vietnam *C. aeruginosa*. The antibacterial assay of MeOH extract of *C. aeruginosa* rhizomes on *S. typhi* and *E. coli* showed moderate activity in 50 ppm with an inhibition zone of 7 mm (inhibition index of 1.17) and 6 mm (inhibition index of 1.00), in 50 ppm, respectively. Curcumenol also exhibited moderate activity in 50 ppm with 8 mm of inhibition zone (1.33 of inhibition index) on *S. typhi* while on *E. coli* showed weak activity in 50 ppm with 4 mm of inhibition zone (0.67 of inhibition index). However, both the methanol extract of *C. aeruginosa* rhizomes and curcumenol were inactive on *B. cereus* and *S. aureus*. It can be concluded that curcumenol played an important contribution to antibacterial activity toward Gram-negative (*S. typhi* and *E. coli*) in *C. aeruginosa* rhizomes.

ACKNOWLEDGMENT

This work was supported by research grant from Department of Chemistry, Institut Pertanian Bogor 2017.

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[RJC-2076/2018]