RJC

RASĀYAN ***J. Chem.***
Vol. 11 | No. 2 |762 - 765 | April - June | 2018
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

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 *TemuHitam,* 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 (^{1}H and ^{13}C) and MS. The preliminary antibacterial assay of methanol extract of *C. aeruginosa* rhizomes on *Salmonella typhi*and *Escherichia coli*showed moderate activitywith 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 moderateactivity 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*.

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 thespecies of *Curcuma* genus (Zingiberaceae), *C.aeruginosa*, locally known as TemuHitam, 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 activitytowardsGram-negative bacteria (*S.typhi* and*E. coli*)and Gram-positive bacteria (*B. cereus* and*S. aureus*)and found that curcumenol is amoderate-active compound isolated from *C. aeruginosa*rhizomes.

EXPERIMENTAL

General Experimental Procedures

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded in $CDCl_3$ using Agilent 500 instrument. Chemical shift references were obtained by addition of TMS. MS spectra were measured

http://dx.doi.org/10.31788/RJC.2018.1122076

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 wascarried out using Si 60 PF_{254} containing gypsum (Merck). Si 60 GF_{254} (Merck) was used for preparative TLC. For TLC analysis, pre-coated silica gel plates (Merck Si 60 GF_{254}, 0.25 mm thickness) and $Ce(SO_4)_2.4H_2O$ 1.5% in H_2SO_4 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 activityassays.Tetracycline was selected as positive control while DMSO was used as negative control. Inhibition index was measured by the following equation (Equation 1).

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

Plant Materials

C. aeruginosa rhizomes were collected from *PusatStudiBiofarmakaTropika* (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:$CHCl_3$ 3:7 as a solvent.

Curcumenol, $C_{15}H_{22}O_2$; white powder; melting point: 117-119.5^oC; *Rf*value: 0.875 in *n*-hexane:$CHCl_3$ 3:7;^{1}H NMR ($CDCl_3$): 5.76 (1H, *s*, H-9), 4.53 (1H,*brs*, -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); ^{13}C NMR ($CDCl_3$): 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 Zingeberaceae 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 activityand 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 inone known guaiane-type sesquiterpenes, curcumenol (Fig.-2).

The structure elucidation of curcumenol was carried out based on one-dimensional NMR (^{1}H and ^{13}C) and also compared with previously reported data[4,8,15]. Based on ^{13}C 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^3bonded to O given the de-shielding δ_C of 85.8-101.7 ppm, and 9 C-sp^3signals in δ_C of 12.0-51.4 ppm. ^{1}H NMR spectra showed that this compound had 22 signals which correspond to 1 H-Csp^2alkenes in $\delta_H$of 5.76 ppm (1H, *s*), 1 H bonded to hydroxyl group in $\delta_H$of 4.53 ppm (1H, *br s*), 2 H-Csp^3bonded to Csp^2 (electron withdrawing group) yielded the de-shielding $\delta_H$of 2.66-2.88 ppm, and the rest were H-Csp^3in $\delta_H$of 1.03-2.00 ppm. The molecular formula concluded from NMR spectral data was $C_{15}H_{22}O_2$ 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
S. typhi (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
E. coli (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
*B. cereus*and *S. aureus* (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)

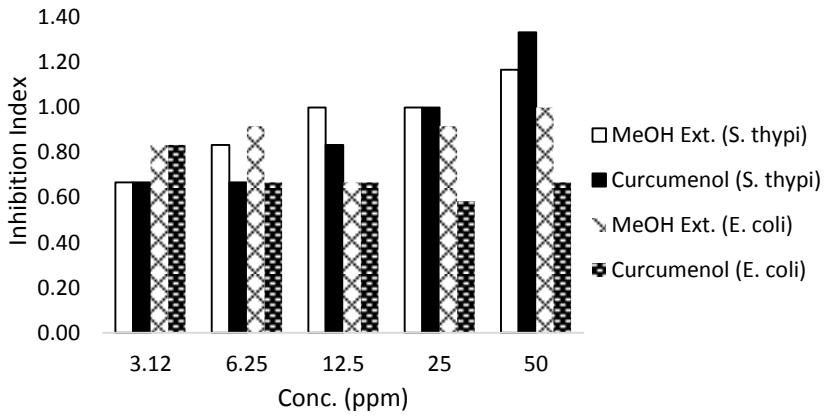

Fig.-1: Inhibition index toward *S. typhi*and *E. coli* from MeOH extract and curcumenol

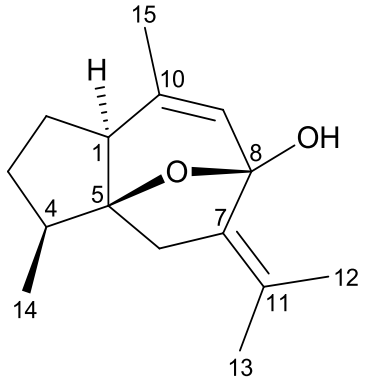

Fig.-2: The structure ofcurcumenol

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 concentrationmostly 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 compoundwas obtained from Malaysian *C. zedoaria* andThailand 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, InstitutPertanian Bogor 2017.

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[RJC-2076/2018]