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.

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. *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).

\[
\text{Inhibition index} = \frac{\text{Inhibition zone of sample}}{\text{Paper disc diameter}}
\]  

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; *Rf* 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*.3,5,10-14 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-sp2 from alkenes double bonds in δc of 122.4-139.3 ppm, 2 C-sp3 bonded to O given the de-shielding δc of 85.8-101.7 ppm, and 9 C-sp3 signals in...
δC of 12.0-51.4 ppm. 1H NMR spectra showed that this compound had 22 signals which correspond to 1 H-Csp3 alkenes in δH of 5.76 ppm (1H, s), 1 H bonded to hydroxyl group in δH of 4.53 ppm (1H, br s), 2 H-Csp2 bonded to Csp2 (electron withdrawing group) yielded the de-shielding δH of 2.66-2.88 ppm, and the rest were H-Csp3 in δH of 1.03-2.00 ppm. The molecular formula concluded from NMR spectral data was C13H2O2 with 5 degree of unsaturated, i.e. 2 alkenes double bonds and 3 cyclics. Therefore, it was no doubtful that the isolated compound was curcumanol. Curcumanol previously isolated from Malaysian C. zedoaria and also Thailand and Vietnam C. aeruginosa1,8,15.

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

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Conc. (ppm)</th>
<th>Inhibition Zone (mm)abc</th>
<th>Activity Classificationabc,d,e,f</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MeOH extract</td>
<td>Curcumanol</td>
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<tr>
<td>S. typhi (Gram -)</td>
<td>3.12</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>6.25</td>
<td>5</td>
<td>4</td>
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<tr>
<td></td>
<td>12.50</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>25.00</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>50.00</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>E. coli (Gram -)</td>
<td>3.12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6.25</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>12.50</td>
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<td></td>
<td>25.00</td>
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<tr>
<td></td>
<td>50.00</td>
<td>-</td>
<td>-</td>
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<tr>
<td>B. cereus and S. aureus (Gram +)</td>
<td>3.12</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>6.25</td>
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<td></td>
<td>12.50</td>
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<td>25.00</td>
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<td>50.00</td>
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</table>

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,d,e,f 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 curcumanol

Fig.-2: The structure of curcumanol

The ability to inhibit bacterial growth toward S. typhi, E. coli, B. cereus, and S. aureus of curcumanol 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.

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