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Research Article

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Curcumenol: A Guaiane-Type Sesquiterpene from Indonesian *Curcuma Heyneana* Rhizome and it's Antibacterial Activity Towards *Staphylococcus Aureus* and *Escherichia Coli*

P Sugita[1*], SO Firdaus[2], A Ilmiawati[2] and DUC Rahayu[2]

[1]*Department of Chemistry, Institut Pertanian Bogor, Kampus IPB Dramaga Bogor, West Java, Indonesia*
[2]*Department of Chemistry, Universitas Indonesia, Kampus UI Depok, West Java, Indonesia*

ABSTRACT

Curcuma heyneana (Zingiberaceae) is one of the zingiberaceous plants indigenous to Java Island, Indonesia which commonly known as Temu Giring. The rhizome of this plant is considered to be useful for treatments of skin diseases, abrasions, and injuries due to the antibacterial compounds contained in this plant. However, there is still scarce information regarding antibacterial compounds contained in C. heyneana rhizomes. In searching of antibacterial compound, isolation of secondary metabolites from Indonesian C. heyneana had been conducted. Curcumenol had been successfully isolated from methanol extract of C. heyneana rhizomes using extraction and various chromatography techniques. This compound had been elucidated using FTIR, GCMS, and NMR (1D and 2D). Curcumenol showed weak antibacterial activity in 50 ppm with inhibition zone of 4 mm and inhibition index of 0.67 towards Escherichia coli (Gram negative) while this compound was inactive towards Staphylococcus aureus (Gram positive).

Keywords: *C. Heyneana*; Curcumenol; Antibacterial; *S. aureus*; *E. coli.*

INTRODUCTION

About 100 *Curcuma* species (Zingiberaceae) are native to South-East Asia, India, and China. The rhizomes of few Curcuma species are widely used as indigenous medicine due to their pharmacological properties which have been identified as antimicrobial [1], anticancer [2,3], antidiarrheal [4], and anti-inflammatory [5].
Curcuma heyneana is one of the zingiberaceous plants indigenous to Java Island, Indonesia. The rhizome of this plant, which is called 'temu giring' in Javanese, are aromatic, pale yellow, and do not contain curcumin, and is considered to be useful for the treatment of skin diseases, abrasions and injuries. It is not only found commonly as one of the main ingredients in traditional Indonesian mixed herbal medicines ("jamu"), but also widely used in the form of a juice prepared from fresh rhizome as an anthelminthic against intestinal worms. Phytochemically, previous investigation had been reported that the major constituent of *C. heyneana* were curcuminoids and sesquiterpenes [6]. More than 25 sesquiterpenes have been isolated from rhizome of *C. heyneana* [7,8] and some biological activities have been reported, such as inhibitor of protein tyrosine phosphatase 1B [7], anti-inflammatory effects [9], cytotoxicity and antibacterial activities [10].

Despite of its numerous medicinal properties and several compounds have been reported, the investigation of antimicrobial activity of sesquiterpenes from *C. heyneana* is still limited. Therefore, this study aimed to evaluate the antibacterial activity of sesquiterpenes from *C. heyneana* rhizomes against *S. aureus* dan *E. coli* using disc diffusion method. There is an urgent need to search for new antimicrobial compounds with diverse chemical structures and novel mechanism of action because there has been an alarming increase in the incidence of infection diseases, as well as the development of resistance to antibiotic in current clinical uses [11].

EXPERIMENTAL SECTION

General Procedures

Si 60 G (Merck) for column packed and Si 60 (0.2-0.5 mm) (Merck) for sample adsorbed were used for vacuum liquid chromatography (VLC), Si 60 PF254 containing gypsum (Merck) was used for Radial chromatography (RC), and Si 60 GF254 (Merck) was used for preparative thin layer chromatography (p-TLC). Pre-coated silica gel plates (Merck Si 60 GF254, 0.25 mm thickness) and $Ce(SO_4)_2.4H_2O$ 1.5% in H_2SO_4 2N as apparition stain reagent were used for TLC analysis. For structure elucidation, FTIR with KBr was used. GCMS was also used with the condition as follows: front inlet using split mode, with initial temperature of 270°C, pressure at 16.38 psi, and helium (He) as carrier gas. Oven condition was set in initial temperature at 60°C and final temperature at 50℃ with flow time of 39.00 minutes. The column size was 60 m × 0.25 mm × 0.25 µm with static flow rate, 0.9 mL/minutes. Ionization at mass spectrometer was done using electrone ionization (EI). MS spectrum had been matched with Wiley 9th library and National Institute of Standard and Technology (NIST) 1D (1H NMR (500 MHz) and 13C NMR (125 MHz)) and 2D (HSQC and HMBC) spectra were recorded in $CDCl_3$ using Agilent 500 instrument. Disc diffusion method was used to measure the antibacterial activity. Tetracycline as a positive control and two bacterias, i.e. *S. aureus* and *E. coli* from Departement of Biology IPB were used for measuring antibacterial activity. Equation 1 was used to measure the inhibition index.

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

Isolation of Curcumenol

C. heyneana rhizomes from Center for Tropical Biopharmaca (Trop BRC), West Java, Indonesia was dried (789.63 g), powdered, and subjected to maceration three times with MeOH at room temperature to yield 121.35 g of crude extract. The crude extract (30 g) then separated with VLC using n-hexane:EtOAc as a solvent and gave seven major fractions (Fr. A-J). Fr. D (798.1 mg) was fractionated using RC with n-hexane:EtOAc as a solvent. Five fractions (Fr. D1-D5) were obtained and Fr. D2 was purified by RC with the same solvent yielding 2 sub-fractions (Fr. D21-D22). Fr. D22 (115 mg) then was purified by p-TLC using n-hexane:DCM:MeOH (7:2:1) as a solvent to give 1 spot dominant (40.2 mg). The compound was identified as curcumenol using FTIR, GCMS, and 1D and 2D NMR.

Evaluation of Antibacterial Activity

The crude extract and curcumenol was examined in antibacterial activity against *S. aureus* and *E. Coli* using disc diffusion methods [12].

Physical Properties and Structure Elucidation of Curcumenol

Yellowish oily, FTIR (cm-1): 3375, 2960, 1660, 1381, 1277, 1060, and 837; GCMS: 14.602 minute retention time; 1H-NMR and 13C-NMR ($CDCl_3$).

RESULTS AND DISCUSSION

The D22 fraction chromatogram yields several peaks from retention time of 12.00-20.00 minutes. The peak with a retention time of 14,60 minute has a high dominant abundance, 96.181% so that other peaks are regarded as minor peaks or impurities (Figure 1). The peak mass spectrum with 14,60 minute retention time was determined possibility of its structure with NIST14.L library and identified as a curcumenol (Figure 2) which have a $C_{15}H_{22}O_2$ molecular formula with m/z 234 (Figure 3) with an estimated quality of 99%.

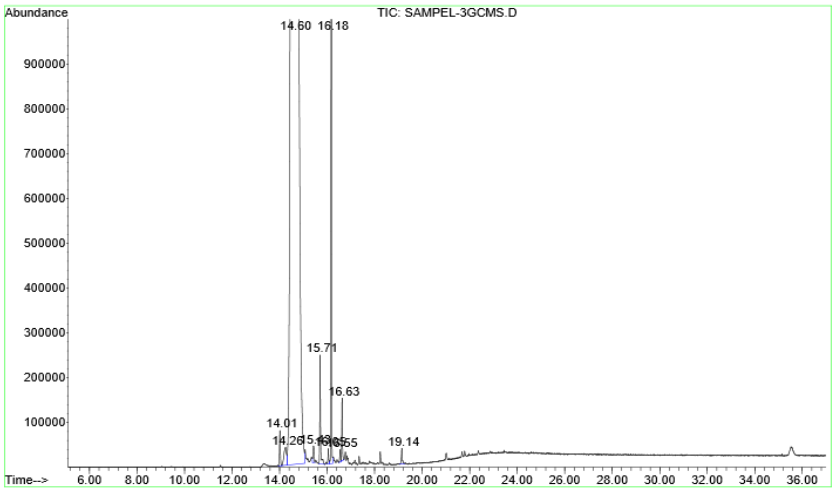

Figure 1: Chromatogram of D22

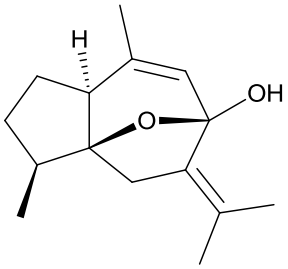

Figure 2: The structure of curcumenol

The identification of the fraction D22 with IR indicates the presence of a methyl group (CH_3), double bond of carbon-carbon, a carbon-oxygen bond with oxygen in functional groups of ether and alcohol. Ether is indicated of forming a furan ring and alcohol as a tertiary alcohol.

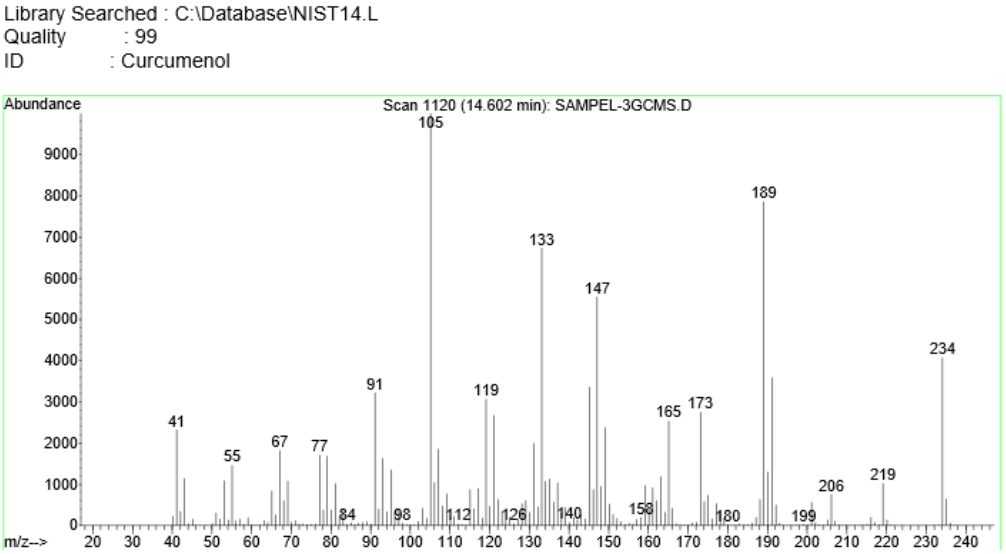

Figure 3: The mass spectrum of D22 peak with an 14.602 minute retention time

Based on 1H NMR spectrum there are presumed to be 4 methyl protons, that is, at the chemical shifts (δH) 1.60 (H-12), 1.67 (H-13), 1.03 (H-14), 1.82 (H-15) which showing integration equal to 3 hydrogen and become a methyl

signal. H-12 and H-13 proton signals exhibit singlet multiplicity, supposedly adjacent to a non-protonated quarterner carbon while proton signals H-14 and H-15 have multiplicity of doblet because they have a neighboring carbon with 1 hydrogen.

Based on the HSQC spectrum (Figure 4), the H-12 proton is correlated with 22.30 ppm (C-12) carbon, H-13 proton correlated with 18.91 ppm (C-13) carbon, H-14 proton correlated with 11.91 ppm (C -14), and the H-15 proton correlated with 21.00 ppm carbon (C-15). The results of the interpretation show that there are 4 methyl carbon in the weak chemical field shift, that is at the chemical shifts (δC) 22.30 (C-12), 18.91 (C-13), 11.91 (C-14), and 21.00 (C-15) ppm.

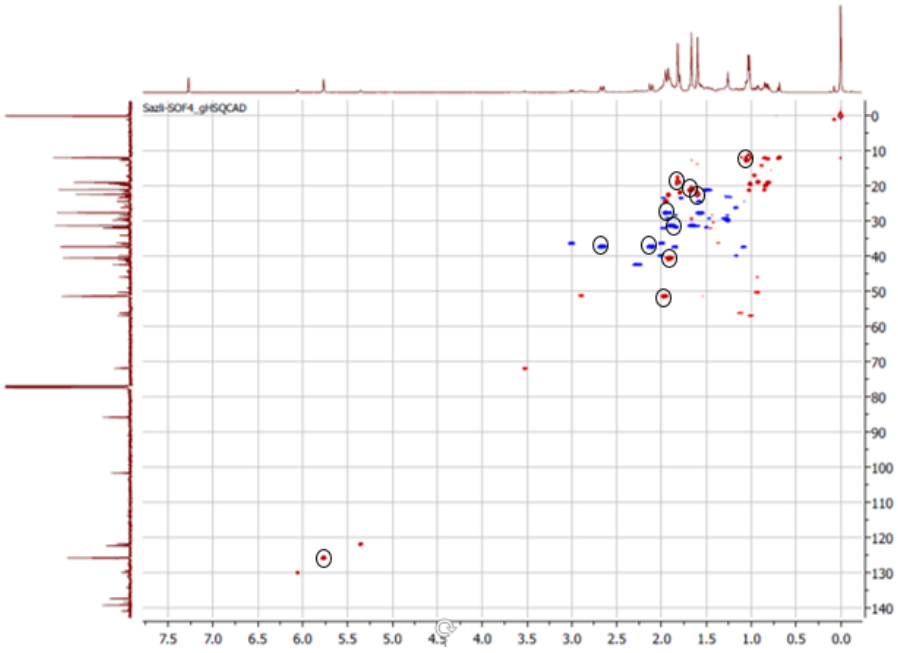

Figure 4: Spectrum 2-dimension HSQC NMR of D22

Based on 13C NMR spectra, there were 15 signals of carbon which correspond to sesquiterpenes derivatives with 5 carbon in the form of a quaternary carbon. Quaternary carbon is identified in very strong fields with a chemical shift (δC) of more than 100 ppm i.e. 101.50 (C-8), 85.67 (C-5), 139.11 (C-7), 137.28 (C-10), and 122.20 (C-11) ppm. The 5 carbon is not correlated with the proton on the HSQC spectrum. The C-7, C-11 and C-10 quaternary carbon is a sp2 dominant carbon characterized by high chemical shift.

The D22 compound is thought to have a bisiklo [5.3.0] hydroazulene basic skeleton and is proved by the presence of quarterner carbon in C-1 and C-5 and there is a correlation of HMBC (Figure 5) between H-9, H-15, H-2, H-3 and C-1 proton so clearly showed the ring characteristics bicyclic of hydroazulene. The binding of methyl C-14 on C-4 tertiary carbon is characterized by HMBC correlation between H-14 and C-3, C-4 and C-5 proton. The binding of methyl C-15 on C-10 is characterized by HMBC correlation between proton H-15 and C-1, C-9 and C-10 carbon. Carbon sp2 C-7 has a 2-propanylidene branch with a double bond between C-7 and C-11. Carbon C-7 has a chemical shift (δC) higher than carbon C-11 because carbon C-7 has a position closer to the oxygen atom so it is more not shielded. The proof of the presence of the 2-propanylidene branch is the HMBC correlation between proton of H-12 and carbon of C-11 and C-13, proton of H-13 and carbon of C-11 and C-12, and methylene protons of H-6 and C-11. With the discovery of a methyl branch on carbon C-4, C-10, and propanylidene on carbon C-7 with a hydroazulene framework shows that the sesquiterpena is guaiane.

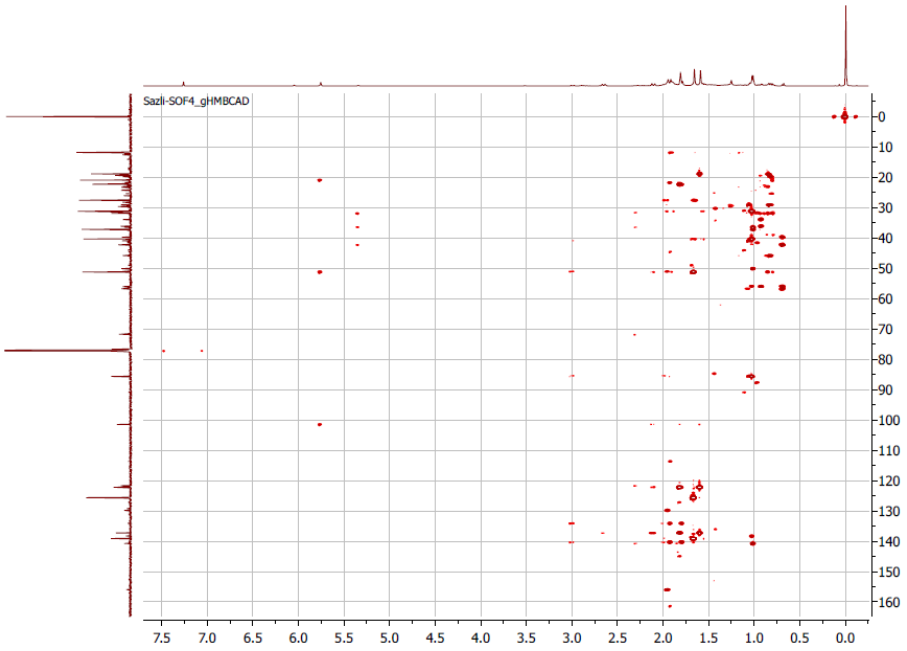

Figure 5: Spectrum HMBC NMR of D22

Based on the IR spectrum indicates functional groups of alcohols and ether and the mass spectrum supports there are two oxygen. The C-8 oxycarbon signals bind to both oxygen into form hemiasetal, the hydroxyl group (alcohol) and the ether forming the furan ring with C-4. In the presence of these bonds make the molecular weight becomes complete, ie 234 g/mol. The correlation of protons with some other neighboring carbon on the two dimension-NMR spectrum of HMBC is presented in Figure 6. The results of 1H NMR spectrum analysis, 13C NMR, and 2 dimensional of NMR (HSQC and HMBC) show that the compound D22 is curcumenol. Then, the NMR D22 spectrum was compared with previous researchers Hamdi et al. [13] and Khine [14] who interpreted curcumenol of *C. zedoaria* from Tawamangu and *C. heyneana* from Bandung Indonesia, respectively (Table 1).

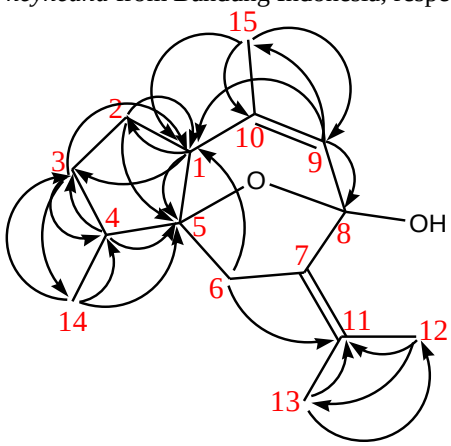

Figure 6: The correlation of protons with some other neighboring carbon for curcumenol on the two dimension-NMR spectrum of HMBC

Table 1: Comparison of NMR spectrum with other reseacher

Carbon number	Fraction D22		Hamdi *et al.* [13]		Khine [14]
	δ_H (Intg, *mult, J*)	**δ_C**	**δ_H (Intg, *mult, J*)**	**δ_C**	**δ_H (Intg, *mult, J*)**
1	1.96 (1H, *m*)	51.25	1.9 (1H, *m*)	51.3	Proton 1−4 1.54−2.00 (6H, *m*)
2	1.97 (2H, *m*)	27.59	1.9/1.9 (1H, *m*)	27.6	
3	1.90 (2H, *m*)	31.22	1.9/1.9 (1H, *m*)	31.2	
4	1.91 (1H, *m*)	40.35	2.62 (2H, *d*, 15.6 Hz)	40.4	
5		85.67		85.8	
6	2.12 (1H, *d*, 16.00 Hz); 2.66 (1H, *d*, 16.00 Hz)	37.22	2.11/2.66 (2H, *d*, 15.4 Hz)	37.2	2.111 (1H, *br d*, 15.4 Hz); 2.657 (1H, *br d*, 15.4 Hz)
7		139.11		139.2	
8		101.50		101.6	
9	5.77 (1H, *s*)	125.64	5.74 (1H, *br s*)	125.7	5.756 (1H, *br s*)
10		137.28		137.2	
11		122.20		122.3	
12	1.60 (3H, *s*)	22.30	1.54 (3H, *s*)	22.4	1.813 (3H, *s*)
13	1.67 (3H, *s*)	18.91	1.61 (3H, *s*)	18.9	1.593 (3H, *s*)
14	1.03 (3H, *d*, 6.25 Hz)	11.91	1.01 (3H, *d*, 6.4 Hz)	11.9	1.022 (3H, *d*, 6.0 Hz)
15	1.82 (3H, *d*)	21.00	1.79 (3H, *s*)	21	1.660 (3H, *s*)

All three data provide a value of chemical shift, integration, multiplicity, and coupling constants with high similarity. Based on these data it can be concluded that the main compound in the fraction of D22 is a curcumenol compound of sesquiterpene type guaiane. These results proved the alleged compound proposed based on GC-MS analysis. Formerly, this compound was obtained from C. zedoaria Tawangmangu Indonesia [13], and also C. aeruginosa Thailand, Vietnam and Indonesia [15-17].

Antibacterial activity of MeOH extract and curcumenol showed weak-moderate activity toward E. coli with inhibition zone of 4-6 mm and 3.5-5 mm (Table 2) in concentration range between 3.12-50 ppm. Both samples were inactive toward *S. aureus*. This antibacterial activity, especially toward Gram-negative bacteria (*E. coli*), enriched the information regarding Curcuma extract having antibacterial activities. Previous studies had been reported that besides *C. heyneana* extract, the others Curcuma extract also had antibacterial activity, such as *C. aregunosa*, *C. zedoaria*, *C. longa*, and *C. xanthorrhiza* [17-24]. The inhibition index of both samples were showed in Figure 7. Figure 7 showed that increasing of concentration will increase the inhibition index.

Table 2: Antibacterial activity of MeOH extract of *C. heyneana* rhizomes and curcumenol toward *S. aureus* and *E. coli*

Bacteria	Conc. (ppm)	Inhibition Zone (mm)[a]	
		MeOH Extract	Curcumenol
S. aureus (Gram positive)	3.12	~0	~0
	6.25	~0	~0
	12.50	~0	~0
	25.00	~0	~0
	50.00	~0	~0
E. coli (Gram negative)	3.12	5	5
	6.25	5.5	4
	12.50	4	4
	25.00	4.5	3.5
	50.00	6	4
[a]Inhibition zone was measured in compared with paper disk diameter (6 mm)			

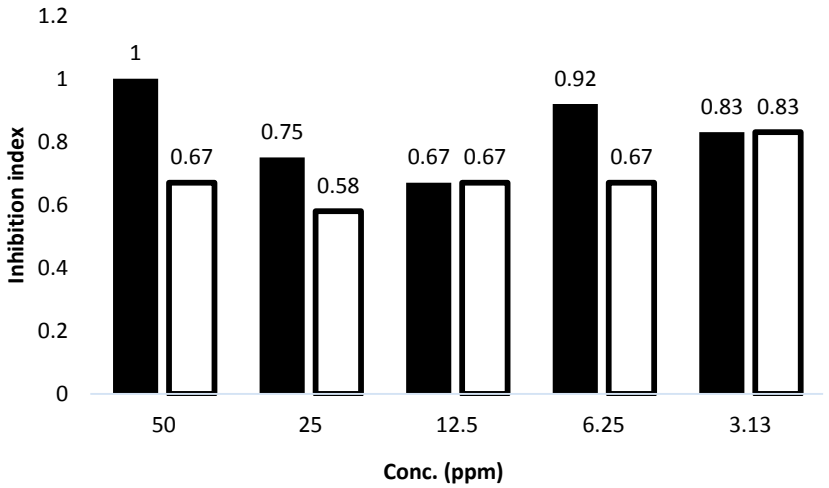

Figure 7: Inhibition index toward *E. coli* (■=MeOH extract, ▭=curcumenol)

CONCLUSION

Curcumenol had been isolated from MeOH extract of Indonesian *C. heyneana* rhizomes. Both the MeOH extract of *C. heyneana* and curcumenol were weak-moderate activity towards *E. Coli* (Gram negative), in contrast, they were inactive on *S. aureus* (Gram positive).

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