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Chemical Constituents and Bioactivity of *Curcuma aeruginosa* Roxb.

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Abstract – Phytochemical study on the rhizomes of *Curcuma aeruginosa* has yielded three sesquiterpenes, which were identified as zedoarol (**1**), curcumenol (**2**) and isocurcumenol (**3**). The structures of the compounds were determined by Infrared Spectroscopy (IR), Nuclear Magnetic Resonance (1D and 2D NMR) and Mass Spectroscopy (MS). The crude extracts and pure compounds obtained were tested against pathogenic microbes and cancer cell lines
Keywords – *Curcuma aeruginosa*, zedoarol, curcumenol, isocurcumenol, bioassay test

Introduction

The scope of our work involves the phytochemical and bioactivity studies of selected *Curcuma* species which belong to Zingiberaceae family. Zingiberaceae plants spread all over the world with 47 genera and 1400 species of perennial herbs. *Curcuma aeruginosa* is recognized as temu hitam in Malaysia. It is a monocotyledonous perennial with glabrous green and purple center bar leaves. The whole plant is about 18 inches tall and has weak aromatic odor (Brouk, 1975). The plant is indigenous to southern Asia but now cultivated in many other tropical regions, such as Malaysia, Indonesia, Thailand, Vietnam, Myanmar and Cambodia (Burkill, 1966). Its rhizomes of has long been used as gastrointestinal remedy and spices in east and southern Asia. This *Curcuma* species is being used by Malay as medicine in childbirth because of its purgative action. The extract of *Curcuma aeruginosa* is also extensively used as a traditional medicine in other countries, especially in Indonesia. It is said that the extract is used as local anesthetics in cold, cough, asthma and other preparation, besides having antispasmodic effect (Kartasapoetra, 1998). Previous investigation have revealed the isolation of several sesquiterpenes from the rhizomes of *Curcuma aeruginosa* collected in Negeri Sembilan, Malaysia and Yakushima island, Japan (Sirat *et al*., 1998; Takano *et al*., 1995). Here we wish to report the isolation and characterization of sesquiterpenes from rhizomes of *Curcuma aeruginosa* collected from Java, Indonesia. Phytochemical investigations on this Zingiberaceous species originated from here have never been reported previously.

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Experimental

General – Melting points (uncorrected) were determined on Kohfler melting points apparatus. Infrared spectra were recorded using KBr disc on Perkin Elmer FTIR spectrophotometer model 1725X. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on JEOL Spectrometer at 500 and 125 MHz, respectively, with tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on an AEI-MS 12 spectrometer. Column chromatography was carried using silica gel (Merck 9385) and Merck silica gel 60 PF_{254} was used for TLC analysis.

Plant material – The plant was collected from Yogyakarta, Indonesia in 1999 and was air-dried prior being used. The plant was identified by Dr. Sugeng Riyanto, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia.

Extraction and Isolation – Ground dried rhizomes of *Curcuma aeruginosa* (1.2 kg) was extracted with petroleum ether and chloroform each for three times at room temperature. The solvents were removed under reduced pressure to give 200 g dark gummy solid of petroleum ether extract and 60 g oily solid of chloroform extract. A

portion of petroleum ether extract (12 g) and chloroform extracts (15 g) were subjected to flash column chromatography separation and eluted stepwise with petroleum ether, mixtures of petroleum ether/chloroform, chloroform, mixtures of chloroform/methanol and methanol to give 60 fractions for petroleum ether extract and 56 fractions for chloroform extract.

Fraction 18 (0.40 g) obtained from separation of petroleum ether extract was rotary-evaporated and the solid obtained was recrystallized in methanol to give colourless leaflet-shaped solid of zedoarol (1) (13 mg), m.p 50 - 52 °C (Shiobara *et al*., 1986, isolated as colorless oil). IR ν_{max} (cm^{-1}, KBr disc) 3540, 2980, 1660, 1452, 1158, 607, 659. EIMS *m/z* (%, relative intensity): 246 (M^+, 35), 231 (3), 213 (5), 203 (6), 188 (19), 175 (94), 161 (39), 147 (22), 133 (17), 119 (73), 105 (25), 91 (40), 77 (29), 43 (100), 41 (72). ^{1}H-NMR δ (400 MHz, $CDCl_3$) 7.07 (1H, br *s,* H-12), 5.16 (2H, br *s,* H-15), 3.83 (2H, *d, J* = 18.1 Hz, H-9), 2.98 (1H, *t*, *J* = 9.3 Hz, H-1), 2.53 (1H, *tq,* H-4), 2.20 (1H, br *s,* OH), 2.16 (3H, *s, J* = 1.4 Hz, H-13), 1.88 (2H, *m,* H-2), 1.46 (2H, *m,* H-3), 1.09 (3H, *d, J* = 6.8 Hz, H-14). ^{13}C- NMR δ (100 MHz, $CDCl_3$) 197.0 (C-6), 158.3 (C-8), 140.8 (C-10), 138.4 (C-12), 122.4 (C-11), 119.8 (C-7), 84.1 (C-5), 50.7 (C-1), 40.3 (C-4), 39.0 (C-9), 30.0 (C-3), 27.2 (C-2), 115.1 (C-15), 14.3 (C-14), 9.5 (C-13).

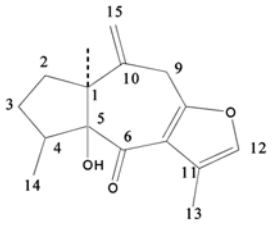
Work-up procedure on fraction 32 (0.92 g) of petroleum ether extract yielded colorless needle-shaped crystals of curcumenol (2) (30 mg), m.p 110 - 112 °C (Firman *et al*., 1988, 114 - 116 °C). IR ν_{max} (cm^{-1}, KBr disc) 3374, 2962, 2934, 1660, 1448, 1222, 982, 880. MS *m/z* (%, relative intensity): 234 (M^+, 41), 219 (6), 206 (7), 189 (61), 173 (22), 165 (14), 147 (52), 133 (60), 119 (29), 105 (100), 91 (30), 79 (18), 67 (22), 43 (36), 41 (67). Fraction 15 (0.55 g) from column chromatography of chloroform extract yielded isocurcumenol (3) as colorless needle-shaped crystals (20 mg), m.p. 142 - 144 °C (Firman *et al*., 1988, m.p.144 - 146 °C). IR ν_{max} (cm^{-1}, KBr disc) 3440, 3066, 1698, 1644, 1146, 1110, 982, 882. MS *m/z* (% intensity): 234 (M^+, 14), 219 (13), 201 (12), 191 (100), 173 (37), 164 (14), 147 (61), 133 (48), 121 (88), 105 (77), 93 (74), 79 (49), 67 (75), 55 (67), 41 (67).

Bioassay – As for bioassay tests, several pathogenic microbial strains including *Pseudomonas aeruginosa, Staphyllococcus aures* and *Bacillus substilis* were used for antimicrobial activity evaluation, whereas T-lymphoblastic leukemia cell line was used for cytotoxic test. The test were carried out according to the methods described previously (McKeen *et al*., 1997).

Results and Discussion

Extraction and separation on the isolates of petroleum ether and chloroform extracts of *C. aeruginosa* have led to the isolation and characterization of three sesquiterpenes, which were identified as zedoarol **(1)**, curcumenol **(2)** and isocurcumenol **(3)**. Compound **3** was isolated for the first time from this plant species. The compounds were characterized using spectroscopic methods and by comparison with the literature.

Compound **1** (13 mg), which was recrystallized from methanol and had a melting point of 50 - 52 °C (Shiobara *et al*., 1986, isolated as colorless oil). The IR spectrum indicated the presence of carbonyl group with strong absorption at 1660 cm^{-1} and broad band at 3540 cm^{-1} due to the presence of hydroxyl group. The EIMS gave a molecular ion peaks at m/z 246 which correspond to the molecular formula of $C_{15}H_{18}O_3$. The ^{1}H-NMR spectrum shows broad signal at δ 2.20 revealing the presence of hydroxyl group and peaks at δ 5.16 and δ 7.07 due to the presence of olefinic protons at H-15 and H-12, respectively. Meanwhile, methyl protons showed as doublet at δ 1.09 (H-14) and singlet at δ 2.16 (H-13). Another doublet signal with *J* value 18.1 Hz at δ 3.83 was due to inequivalent methylene protons of H-9. Methylene protons at H-2 and H-3 appeared as multiplets at δ 1.88 and δ



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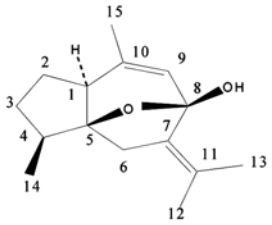

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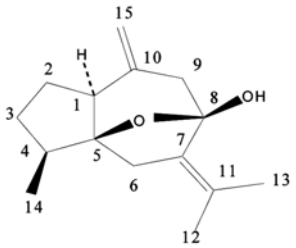

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1.46, respectively. ^{13}C-NMR spectrum indicated the presence of 15 carbons of which six are quaternary carbons at δ 84.1, δ 197.0, δ 119.8, δ 158.3, δ 140.8 and δ 122.4 which were assigned to C-5, C-6, C-7, C-8, C-10 and C-11, respectively. Three methine carbons observed at δ 50.7, δ 40.3 and δ 138.4 were assigned to C-1, C-4 and C-12, respectively. The presence of four methylene carbons were indicated at δ 27.2 (C-2), δ 30.0 (C-3), δ 39.0 (C-9) and δ 115.1(C-15). In addition, two methyl carbons were observed at δ 9.5 (C-13) and δ 14.3 (C-14). The presence of carbonyl group was indicated at δ 197.0 (C-6). The above spectral data together with the molecular formula have identified the compound as zedoarol **(1)**. The ^{13}C NMR and ^{1}H NMR assignment were also compared with previous report (Shiobara *et al*., 1986).

Compound **2** was recrystallized with methanol and decomposed at 110 - 112 °C (Firman *et al*., 1988, m.p. 114 - 116 °C). The EIMS gave a molecular ion peaks at m/z 234 which correspond to the molecular formula of $C_{15}H_{22}O_2$. The IR spectrum exhibited absorption band at 3374 cm^{-1} which correspond to hydroxyl group, while peaks at 1660 cm^{-1} and 1448 cm^{-1} were due to double bond. The presence of C-O bond was indicated by the peak at 1222 cm^{-1}. The ^{1}H-NMR spectrum indicated the presence of four methyl groups at δ 1.59, δ 1.66, δ 1.03 and δ 1.81 (H-12, H-13, H-14 and H-15). Two methylene groups (H-2 and H-3) and two methine protons (H-1 and H-4) occur at higher field between δ 1.80 - δ 2.00, while nonequivalent methylene protons (H-6) resonated as dublets at δ 2.10 and δ 2.65. Olefinic proton of H-9 appeared at δ 5.76, while hydroxyl group appeared as broad peak at δ 3.05. The ^{13}C-NMR spectrum accounted for 15 signals correspond to the presence of 15 carbons. The presence of four methyl carbons, three methylene carbons and three methine carbons was supported by DEPT spectral data. All these assignment were further supported by HMQC and HMBC correlation spectra (Table 1). The HMQC correlation revealed that the proton signal for H-1 showed a cross peak with carbon signal at δ 52.3 (C-1), whereas proton signals at δ 2.10 and δ 2.65 due to inequivalent H-6 protons correlated to carbon signal at δ 38.2 (C-6). The HMBC spectrum revealed that the carbon signal at δ 19.7 (C-13) was seen to correlate with proton signal at δ 1.59 (H-12). Besides that, another carbon signal at δ 21.8 (C-15) correlated with proton signal at δ 5.76 (H-9), while C-2 (δ 28.2) was simultaneously coupled with H-3 (δ 1.80 - δ 2.00) and H-1 (δ 1.80 - δ 2.00). In addition, signal at δ 32.2 (C-3) was shown to correlate to proton signals at δ 1.80 - 2.00 (H-2) and (H-4), and δ 1.03 (H-14). The location of hydroxyl group at C-8 (δ 102.5) was supported by the cross peak between the C-8 (δ 102.5) and olefinic proton of H-9 (δ 5.76). The presence of methyl group attached to C-4 was suggested with correlation of H-14 (δ 1.03) with C-4 (δ 41.3), C-5 (δ 86.1) and C-3 (δ 32.2). All the spectral evidences mentioned above suggested that compound **2** was curcumenol (Firman *et al*., 1988).

Compound **(3)** was obtained as colorless needle-shaped crystal (20 mg) with melting point of 142 - 144 °C (Firman *et al*., 1988, 144 - 146 °C). This compound was isolated from column chromatography separation of chloroform extract. The IR spectrum showed a strong absorption at 3440 cm^{-1} which suggests the presence of hydroxyl group. Strong C = C stretching absorption peak was observed at 1698 cm^{-1}, while C-O stretching peak was observed at 1110 cm^{-1}. The EIMS showed a molecular peak at *m/z* 234 suggesting a molecular formula of $C_{15}H_{22}O_2$. The ^{1}H NMR spectrum indicated the presence of three methyl groups at δ 1.62, δ 1.80, δ 1.01 (H-12, H-13, H-14). Two pairs of triplets at δ 4.74 and δ 4.78 were due to inequivalent olefinic protons of H-15, while a broad peak at δ 2.92 was due to hydroxyl group. Four methylene protons of H-2, H-3, H-6 and H-9 appeared as multiplets at δ 1.50 - 2.70. HMQC spectrum of compound (3) indicated that methylene group protons of H-3, H-4, H-9 and H-15 were inequivalent. The pattern of its ^{13}C-NMR spectrum (Table 1) was similar to those of curcumenol (**2**) except for the signal of C-9 and C-15. The presence of these two signals was substantiated by HMQC spectrum with the correlation of C-9 (δ 36.1) with H-9 (δ 1.93-2.00 and δ 2.50-2.60) and C-15 (δ 112.2) with H-15 (δ 4.74 and δ 4.78). The assignments of ^{1}H and ^{13}C chemical shifts were substantiated by HMQC and HMBC correlation spectra (Table 1). The HMBC spectrum showed that peak at δ 133.7 (C-7) correlated with the proton signal at δ 1.62 (H-12), δ 1.80 (H-13), and δ 1.93-2.00 (H-6). Similarly, carbon peak at δ 126.9 (C-11) also exhibited correlations with proton signal at δ 1.62 (H-12) and δ 1.80 (H-13). The complete HMBC assignment are listed in Table 1. The spectral evidences described above suggested that compound 3 was isocurcumenol, which was isolated previously from *Curcuma heyneana* (Firman *et al*., 1988).

As for bioassay investigation, cytotoxic test of chloroform extract from *C. aeruginosa* showed strong activity against cancer cell lines with IC_{50} value 6 μg/ml (Table 2). In the antimicrobial test, $CHCl_3$ extract of the plant exhibited moderate activity against *Pseudomonas aeruginosa* (60690), and weakly susceptible to *Bacillus substillis* (B29), *Bacillus substillis* mutant (B28) and

Table 1. ^{1}H-, ^{13}C-NMR, and HMBC correlations spectral data of compounds **2** and **3**

Carbon number	Compound **2** δ^{1}H (ppm)	δ^{13}C (ppm)	HMBC correlations	Compound **3** δ^{1}H (ppm)	δ^{13}C (ppm)	HMBC correlations
1	1.46-2.98 (6H, *m*)	52.3	H-2, H-3, H-6, H-9, H-15	2.18 (1H, *t*)	52.7	H-4, H-15
2		28.2	H-1, H-3	1.66-1.80 (2H, *m*)	28.3	
3		32.2	H-2, H-4, H-14	1.93-2.00 (1H, *m*)	30.7	H-14
4		41.3	H-3, H-14	1.50-1.60 (1H, *m*)		
				2.50-2.60 1H, *m*)	41.6	H-14
				2.60-2.70 (1H, *m*)		
5		86.1	H-4, H-6, H-14		87.0	H-14
6	2.65(2H, *d*, *J* = 15.5Hz) 2.10(2H, *d*, *J* = 15.5Hz)	38.2	H-4	1.93-2.00 (4H, *m*)	38.9	
7		138.3	H-6 ,H-13, OH		133.7	H-12, H-13, H-6
8		102.5	H-9, OH		103.9	H-9
9	5.76 (1H, *s*)	128.1	H-15, OH	1.93-2.00(1H, *m*) 2.50-2.60 (1H, *m*)	36.1	
10		137.2	H-15		145.0	H-9
11		122.2	H-12, H-13, H-6		126.9	H-12, H-13
12	1.59 (1H, *s*)	22.3	H-13	1.62 (3H, m)	22.5	H-13
13	1.66 (1 H, *s*)	19.7	H-12	1.80 (3H, *m*)	18.9	H-12
14	1.03 (3H, *d*, *J* = 6.4Hz)	12.6		1.01 (3H, *d*, *J* = 6.4Hz)	12.4	
15	1.81 (1H, *s*)	21.8	H-9	4.78 (1H, *t*, *J* = 2.1Hz) 4.74 (1H, *t*, *J* = 2.1Hz)	112.2	H-9
OH	3.05 (1H, br *s*)		H-8, H-9, H-7	2.92 (1H, br *s*)		

Table 2. Antibacterial and cytotoxic activities of extracts and pure compounds from *Curcuma aeruginosa*

plant	extracts / pure compounds	cytotoxic activity IC_{50} (μg/mL)	antimicrobial activity; diameter of zone of inhibition (mm)			
			MRSA	60690	B29	B28
Curcuma aeruginosa	petroleum ether	> 30	–	–	–	–
	$CHCl_3$	6	9	10	6.5	7
	MeOH	> 30	–	–	–	–
	zedoarol (1)	n.t	n.t	n.t	n.t	n.t
	curcumenol (2)	> 30	–	–	–	–
	isocurcumenol (3)	> 30	12	–	–	10

– : no activity.
MRSA : *Staphylococcus aures*
B29 : *Bacillus substillis*
n. t : not tested
60690 : *Pseudomonas aeruginosa*
B28 : *Bacillus substillis mutant*

Staphylococcus aures (MRSA). However, petroleum ether and methanol extracts were inactive against all the pathogenic microbes and cancer cell lines used in the tests. Two pure compounds from *Curcuma aeruginosa* were also tested against antimicrobial activity. The results showed isocurcumenol exhibited moderate activity against *Bacillus substillis and Pseudomonas aeruginosa,* but was not active activity against *Bacillus substillis mutant* and *Staphylococcus typhimurium*. Meanwhile, curcumenol did not show any activity against all pathogenic bacteria.

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