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TERPENOIDS FROM THE STEM BARK OF *JATROPHA* PLANTS AND THEIR BIOLOGICAL ACTIVITIES

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Abstract

Three terpenoids, including two diterpenes (curcusone B and jatrophone) and a triterpene (stigmasterol) have been isolated from the stem bark of *Jatropha* plants. Curcusone B and stigmasterol were isolated from *J. curcas*, meanwhile jatrophone and stigmasterol were from *J. gossypifolia*. The biological activities of these compounds have been evaluated toward bacteria, fungi and tumour cells. Isolation was carried out in vacuum liqiud cromatography (VLC) technique with silica gel as an adsorben and some solvents as eluents. The compound structures were determined by spectroscopic methodes i.e. UV-vis, FTIR, NMR (1-D, 2-D) and were then compared based on their spectroscopic data with similiar data from literatures. The biological properties of these compounds were evaluated against four strains of bacteria (*Acetobacter sp*., *Eschericia coli*, *Staphylococcus aureus*, and *Streptococcus sp.*), 4 strains of fungi (*Aspergilus niger*, *Penicillium sp.* (grey), *Penicillium sp.* (white) and *Rhizopus sp.*) and murine leukemia P-388 cells. The results showed that cytotoxic property of curcusone B towards murine leukemia P-388 cells is better than jatrophone and stigmasterol which are IC_{50} = 0.57 μg/mL (1.93 μM) for curcusone B and IC_{50} > 100 μg/mL for jatrophone and stigmasterol. Meanwhile, activities against bacteria, jatrophone is better than curcusone B and stigmasterol. Jatrophone is the most active against *S. aureus* (bacteria) with growth inhibition zone 36 mm and *A.niger* (fungi) is 44 mm. Further study indicated that jatrophone was bacteriostatic against *S. aureus*.

Keywords: biological activities, Curcusone B, Jatropha, jatrophone, stigmasterol

1. Introduction

Jatropha curcas and *J. gossypyfolia* (Euphorbiaceae) are known as traditional medicines. Study on the biological activities of extracts of *J. curcas* and *J. gossypyfolia* showed interesting potencies. Stem bark, seed, and leaves were active toward some microbes [1]. In Indonesia, *J. curcas* is used as a cure of eczema, gonorrhea and the dandruff [2]. In addition, in China, grain`s crop is used to treat wounds and skin disorders [3], in Nigeria, *J. curcas*`s fruits are utilized for treating dibetes mellitus [4], and in Comoro Island (Africa), leaves of this plant are used for a malarial drug [5]. Moreover, stem bark and root extracts of these plants showed potency as an antibacteria (*Acetobacter sp*., *Eschericia coli*, *Staphylococcus aureus*, and *Streptococcus sp.*), and antifungal (*Aspergilus niger*, *Penicillium sp.* (grey), *Penicillium sp.* (white) and *Rhizopus sp.*) [6]. Extract of *J. gossypyfolia* is also employed as antibiotic and anti-fertility. In India, the plant extract was toxic against some microbes i.e. *Schistosoma incognitum, S. nasale, Orientobilharzia dattae, Fasciola hepatica, and F. gigantica* [7], *E. coli, Salmonella typhii, Pseudomonas aeruginosa, Bacillus aureus, Klebsiella aerogenes, Proteus vulgaris, dan Candida albicans* [8], and the fresh latex for treating of skin burn [9]. In addition, root of *J. curcas* proved as an anti inflammatory agent and diarrhea [10]. The leaves were toxic against the larva of *Culex quinquefasciatus* and the resin has a coagulant potency [11].

Phytochemical study of *J. curcas* have successfully identified some chemical contents such as coumarino-lignoid from the stem bark [12], dinorditerpene [13], curcusone A-D from the roots [14], and jatrophalactone, jatrophalone, jatrophadiketone [15]. Meanwhile, phytochemical study of *J. gossypifolia* have successfully identified jatrophone [16] and cleomiscosin A [17].

However, information of activities of those compounds toward microbes and cancer cell lines are still limited. This paper will report isolation, structure determination, and activities of curcusone B (**1**), stigmasterol (**2**) and jatrophone (**3**) toward bacteria (*Acetobacter sp*., *E. coli*, *S. aureus*, and *Streptococcus sp.*), fungal (*Aspergilus niger*, *Penicillium sp.* (grey), *Penicillium sp.* (white) and *Rhizopus sp.*), and murine leukemia P-388 cells.

2. Experiment

General experiment procedure. Isolation used vacuum liquids chromatography methods (VLC). VLC was carried out by using Merck Si-gel 60 GF_{254}, and TLC analysis on pre-coated Si-gel plates (Merck Kieselgel 60 F_{254}, 0.25 mm). UV and IR spectra were measured with Cary Varian 100 conc. and Perkin-Elmer Spectrum One FT-IR Spectrophotometer, respectively. ^{1}H and ^{13}C NMR spectra were recorded with a JEOL ECP 500 spectrometer, operating at 500 MHz (^{1}H) and 125 MHz (^{13}C), worked at LIPI Serpong.

Sample of the stem bark of *J. gossypifolia* and *J. curcas* were collected from Pusat Koleksi dan Pengembangan Tanaman Obat Tradisional Masyarakat Sulawesi Tenggara "Arboretum Prof. Mahmud Hamundu" Universitas Haluoleo in January 2009. The plant was identified by Herbarium Bogoriense, Bogor Indonesia, and a voucher specimen has been deposited at the herbarium. Microbes were used in the research that are against bacteria (*Acetobacter sp*., *E. coli*, *S. aureus*, and *Streptococcus sp.*), and fungi (*A. niger*, *Penicillium sp.1*, *Penicillium sp.2* and *Rhizopus sp.*). Culture of bacteria and fungi were obtained from PAU Biotechnology UGM.

Isolation of compounds from stem Barks of *J. curcas*. Powder of stem bark of *J. curcas* (1,0 kg) was macerated by methanol (MeOH) 3 x 3 L for 3 x 24 hs. Methanol macerate was concentrated by vacuum rotary evaporator up to get a dark brown gum (100 g). A part of methanol extract (50 g) was fractionated by VLC using a column Φ 10 cm, adsorben: Si-gel (150 g) and mixture of ethylacetate:*n*-hexane (40-100%, MeOH 100%) as eluent, to give 4 fractions i.e. F1 (5.1 g), F2 (18.0 g), F3 (14.3 g), and F4 (10.2 g), respectively. F2 was refractionated using VLC with a column Φ 5 cm, adsorben: Si-gel (70 g) and mixture of ethylacetate: n-hexane (30-100%, MeOH 100%) as eluent, provide 5 fractions i.e. F1 (1.2 g), F2 (3.0 g), F3 (4.8 g), F4 (2.2 g) and F5 (5.1 g). Moreover, purification of F3 got a yellow crystal (**1)** (300 mg) and from F4 got a white crystal (**2)** (60 mg).

Isolation of compounds from stem Barks of *J. gossypifolia*. The same method as the isolation of curcusone B from stem barks of *J. curcas*, from the powder of stem barks of *J. gossypifolia* (1.0 kg) has been isolated two compounds including a white crystal (**2**) (33 mg) and a white crystal **(3**) (240 mg).

Structure determination of pure compounds. The structure of pure compounds were by using spectroscopy methods including UV-vis, FTIR, NMR 1-D (^{1}H and ^{13}C) and NMR 2-D (HMQC, HMBC and H-H COSY).

*Compound **1.*** A yellow crystal compound, melting point (m.p.) of 128-129 ^{o}C, $[\alpha]_D^{20}$ -543^{o} (*c* 0.1 MeOH), UV-Vis (MeOH) λ_{maks} (log ε) 201 (5.36), 257 nm (3.67). IR spectra (KBr) showed at $\tilde{\upsilon}_{maks}$ (cm^{-1}) 3076 (C*sp*^{2}–H), 2956 (C*sp*^{3}–H), 2928 (C-C alkyl), 1711 (C=O ketone), 1657 (C=O ketone), and 1641, 1445 (C=C). Spectra of ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H (ppm) 5.84 (1H, *d*, *J*=4.9 Hz, H-7), 4.78 (2H, *d*, *J*= 7.9 Hz, H-16a/b), 4.71 (1H, *s*, H-18a), 4.17 (1H, *s*, H-18b), 3.28 (1H, *ddd*, *J*=15, 10, 5 Hz, H-3a), 3.11 (1H, *d*, *J*=15 Hz, H-2), 2.57 (1H, *ddd*, *J*=16.2, 10, 5 Hz, H-8), 2.48 (1H, *m*, H-9), 2.39 (1H, *dt*, *J*=10.8, 6.8, 4.25 Hz, H-12a), 2.34 (1H, *ddd*, *J*=11.5, 10, 5.2 Hz, H-14), 2.24 (1H, *ddd*, *J*=11.5, 10, 3.5 Hz, H-12b), 2.13 (1H, *dt*, *J*=15.2, 6.8, 3.7 Hz, H-3b), 1.85 (1H, *ddd*, *J*=14.8, 10, 4.5 Hz, H-13a), 1.81 (3H, *s*, H-17), 1.55 (3H, *s*, H-20), 1.42 (1H, *ddd*, *J*=11.2, 4.2, 2.5 Hz, H-13b), and 1,17 (3H, *dd*, *J*=15.5, 7.35 Hz, H-19). Spectra of ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C (ppm) 212.1 (C-1), 198.4 (C-5), 158.5 (C-10), 148.9 (C-15), 148.7 (C-11), 146.9 (C-4), 140.9 (C-6), 136.6 (C-7), 113.3 (C-16), 108.2 (C-18), 51.8 (C-14), 45.9 (C-2), 43.7 (C-8), 39.7 (C-9), 36.5 (C-12), 36.3 (C-3), 34.5 (C-13), 19.5 (C-17), 18.8 (C-20), and 14.6 (C-19).

*Compound **2.*** A white crystal compound, m.p. 169-171 ^{o}C. Spectra of IR spectra (KBr) showed at $\tilde{\upsilon}_{maks}$ (cm^{-1}) 3425 (OH), 2935 and 2866 (C*sp*^{3}–H), 1053 (C-O). Spectra of ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H (ppm) 1.82 (1H, *m*, H-1a), 1.15 (1H, *m*, H-1b), 1.95 (1H, *m*, H-2a), 1.85 (1H, *m*, H-2b), 3.35 (1H, *m*, H-3), 2.27 (1H, *m*, H-4a), 2.22 (1H, *m*, H-4b), 5.35 (1H, *br d*, H-6), 1.93 (2H, *m*, H-7), 1.49 (1H, *m*, H-8), 0.91 (1H, *br d*,H-9), 1.47 (2H, *m*, H-11), 2.02 (1H, *m*, H-12), 0,97 (1H, *m*, H-14), 1.54 (2H, *m*, H-15), 1.27 (1H, *m*, H-16), 1.08 (1H, *m*, H-17), 0.84 (1H, *br d*, H-18a), 0.79 (1H, *br d*, H-18b), 0.67 (1H, *br s*,H-18c), 1.00 (3H, *br s*, H-19), 1.97 (1H, *m*, H-20), 1.00 (3H, *br s*, H-21), 5.15 (1H, *dd*, H-22), 5.02 (1H, *dd*, H-23), 0.91 (1H, *br d*, H-24), 1.66 (1H, *m*, H-25), 1.00 (1H, *br s,* H-26a), 0.81 (2H, *br d*, H-26b), 0.91 (1H, *br d*, H-27a), 0.81 (1H, *br d*, H-27b), 0.69 (1H, *br s*, H-27c), 1.44 (2H, *m*, H-28), 0.84 (1H, *br d*, H-29a), 0.79 (1H, *br d*, H-29b), and 0.67 (1H, *br s*, H-29c).

Spectra of ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C (ppm) 37.4 (C-1), 31.8 (C-2), 71.9 (C-3), 42.5 (C-4), 141.9 (C-5), 121.9 (C-6), 32.1 (C-7), 32.1 (C-8), 50.3 (C-9), 36.7 (C-10), 21.2 (C-11), 39.9 (C-12), 42.5 (C-13), 56.9 (C-14), 24.4 (C-15), 28.4 (C-16), 56.2 (C-17), 12.0 (C-18), 21.3 (C-19), 40.7 (C-20), 21.3 (C-21), 138.5 (C-22), 129.4

(C-23), 51.4 (C-24), 31.1 (C-25), 19.2 (C-26), 19.0 (C-27), 26.3 (C-28) and 12.2 (C-29).

*Compound **3**.* A white crystal compound, m.p. 152-153 ^{o}C. Spectra of FTIR (KBr) $\tilde{\upsilon}_{maks}$ (cm^{-1}) 3283 (OH), 2961, 2929 (Csp^3-H), 1690, 1654 (C=O), 1619 (C=C) dan 1292 (C-O). Spectra of ^{1}H NMR (CD_3OD, 500 MHz) δ_H (ppm) 2.20 (1H, *dd*, *J*=7.95; 5.82 Hz, H-1a), 1,79 (1H, *dd*, *J*=7.95; 7.92 Hz, H-1b), 2.98 (1H, *bm*, *J*=2.45; 2.15 Hz, H-2), 5.76 (1H, *d*, *J*=1.2 Hz, H-3), 5.77(1H, *d*, *J*=1.2 Hz, H-5), 5.98 (1H, d, J=15,9 Hz, H-8), 6.60 (1H, d, J=16.5 Hz, H-9), 3.04 (1H, *d, J*=15.3 Hz, H-11a), 2.50 (1H, *d*, *J*=15.3 Hz, H-11b), 1.1 (3H, *d*, *J*=6.75 Hz, H-16), 1.86 (3H, *d*, *J*=1.25 Hz, H-17), 1.26 (3H,*s,* H-18), 1.37 (3H, *s,* H-19), 1.72 (3H, *s,* H-20).

Spectra of ^{13}C NMR (CD_3OD, 500 MHz) δ_c (ppm) 43.4 (C-1), 39.7 (C-2), 124.5 (C-3), 138.8 (C-4), 148.3 (C-5), 143.9 (C-6), 204.1 (C-7), 129.4 (C-8), 162.4 (C-9), 38.0 (C-10), 42.0 (C-11), 187.0 (C-12), 113.9 (C-13), 206.3 (C-14), 101.5 (C-15), 19.7 (C-16), 20.9 (C-17), 30.5 (C-18), 27.2 (C-19) and 6.0 (C-20).

Biological activities test. The antifungal and antibacterial tests were conducted by the agar dilution method using the general procedure outlined by Thakurta [18]. The cultural concentration of bacteria was (*Streptococcus sp*. = 3.4 x 10^7 cfu/mL, *Acetobacter sp*. = 3.5 x 10^7 cfu/mL., *E. Coli* = 4.2 x 10^8 cfu/mL dan *S. Aureus*= 3.2 x 10^7 cfu/mL), and fungi concentrations were *A. niger* = 3.5 x 10^5 cfu/ml, *Rhizopus sp*. = 3.1 x 10^5 cfu/mL, *Penicillium sp*.1 = 2.4 x 10^4 cfu/mL and *Penicillium sp*.2 = 2.8 x 10^4 cfu/mL. The cytotoxic property toward murine leukemia P-388 cells was evaluated using Alley methods [19].

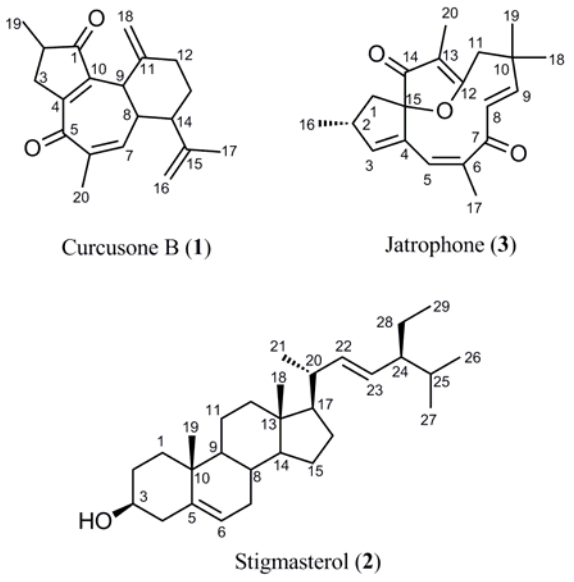

Figure 1. Structure of Terpenoids from *Jatropha* Plants

3. Results and Discussion

In this study, three compounds were isolated from *Jatropha* plants, are known compounds. Thus, the certainty of the isolated compound structures can be done by comparing the spectroscopic data of the isolated compounds with relevant data which have been published (references).

Table 1. Comparison of Spectroscopy Data of Isolate (1) with Curcusone B (1*) [14]

No. C	Isolate (1)		Curcusone B (1*)	
	δH (ppm) (∑H, *m*, J)	δC (ppm)	δH (ppm) (∑H, m, *J*)	δC (ppm)
1	-	212.1	-	211.9
2	2.47 (1H, *m*)	39.7	2.47 (1H, *ddd*, 7.4, 7.4, 3.3)	39.6
3	2.13 (1H, *dt*, 18.3, 3.7, 3.7)	36.3	2.13 (1H, *ddd*, 18.7, 3.4, 3.4)	36.2
	3.28 (1H, *ddd*, 17.8, 7.4, 2.4)		3.29 (1H, *ddd*, 18.7, 7.4, 2.3)	
4	-	146.9	-	146.8
5	-	198.4	-	198.5
6	-	140.9	-	140.8
7	5.84 (1H, *d*, 4.9)	136.6	5.84 (1H, *d*, 5.2)	136.5
8	2.57 (1H, *ddd*, 11.3, 4.3, 4.9)	43.7	2.56 (1H, *m*)	43.6
9	3.12 (1H, *d*, 12.9)	45.9	3.12 (1H, *m*)	45.8
10	-	158.5	-	158.4
11	-	148.7	-	148.6
12	2.24 (1H, *ddd*, 12.9, 12.9, 4.3)	36.5	2.23 (1H, *ddd*, 12.7, 12.7, 4.6)	36.5
	2.39 (1H, *dt*, 12,4, 4.3, 4.3)		2.39 (1H, *ddd*, 12.7, 4.4, 4.4)	
13	1.42 (1H, *dddd*, 12.8, 12.8, 12.8, 4.3)	34.5	1.44 (1H, *dddd*, 12.5, 12.5, 12.5, 4.4)	34.4
	1.85 (1H, *m*)		1.85 (1H, *m*)	
14	2.32 (1H, *ddd*, 11.9, 11.9, 3.7)	51.8	2.32 (1H, *ddd*, 12.5, 12.5, 3.8)	51.7
15	-	148.9	-	148.8
16	4.71 (1H, *s*)	113.3	4.72 (1H, *s*)	113.2
	4.17 (1H, *s*)		4.17 (1H,*s*)	
17	1.56 (3H, *s*)	19.5	1.56 (3H, *s*)	19.4
18	4.79 (1H, *s*)	108.2	4.79 (1H, *s*)	108.1
	4.81 (1H, *s*)		4.80 (1H, *d*, 2.3)	
19	1.17 (3H, *d*, 7.4)	14.6	1.17 (3H, *d*, 7.4)	14.6
20	1.81 (3H, *s*)	18.8	1.81 (3H, *dd*, 2.3, 2.3)	18.7

[1] (^{1}H NMR: 500 MHz, ^{13}C NMR: 125 MHz)
[1*] (^{1}H NMR: 400 MHz, ^{13}C NMR: 100 MHz) [14]

Compound **1** was isolated as a yellow crystal compound with m.p. 128-129 ^{o}C. UV spectra showed that peaks at λ_{maks} (log ε) 201 nm (5.36), and 257 nm (3.67) indicated a conjugated chromofore alkene-carbonyl. It is supported by FTIR spectra at 3076 cm^{-1} (streching =C–H, Csp^2-H), 2956, 2928 cm^{-1} (Csp^3–H), and peaks at 1711 and 1657 cm^{-1} for two units of carbonyl (C=O), and peaks at 1641 cm^{-1} for =C–C=O.

The presence of functional groups is confirmed by data of NMR 1-D (^{1}H and ^{13}C-NMR). Spectra of ^{13}C NMR showed 20 signals of carbon atoms, consisting of 10 aliphatic carbons, 8 carbon atoms sp^2 (C-4,6,7,10,11,15,16,18), and 2 carbon atoms sp^2 carbonyl, C=O (C-1, C-5). Meanwhile ^{1}H NMR spectra showed 16 signals representing 24 aliphatic protons. Based on these data, the isolate has molecular formula $C_{20}H_{24}O_2$ with DBE 9. The data is suitable for curcusone B (**1**). Confirmation of the structure was carried out by NMR-2D (HMQC, HMBC, and H–H COSY) and based on comparison of spectroscopic data (^{1}H and ^{13}C NMR) isolate with spectroscopic data of library [14], see in Table 1. The data indicated highly sameness parameters between compound **1** and curcusone B. Thereby, can be concluded that compound **1** is curcusone B. Structure determination of compound **2** and **3** were carried out by following the same steps as structure elusidation of compound **1** (curcusone B), so compound **2** and compound **3** were sigmasterol [18] and jatrophone [16], respectively.

Biological activities of curcusone B, stigmasterol and jatrophone were analyzed toward four strains of bacteria (*Acetobacter sp*., *E. coli*, *S. aureus*, and *Streptococcus sp.*), and four strains of fungi (*A. niger*, *Penicillium sp.1*, *Penicillium sp.*2 and *Rhizopus sp.*) and murine leukemia P-388 cells. The data is presented in Table 2 and Table 3.

Table 2. Biological Activities of Curcusone B Against Bacteria and Fungi

Test		Diameter of Inhibition zone (mm)				
		1	2	3	4	5
Bacteria	*Streptococcus sp.*	29	8	32	8	17
	Acetobacter sp.	6	2	24	2	15
	E. coli	2	1	22	1	14
	S. aureus	3	1	36	1	19
Fungi	*A. niger*	16	5	44	5	-
	Penicillium sp. 1	15	4	14	4	-
	Penicillium sp. 2	14	3	18	3	-
	Rhizopus sp.	8	1	11	1	-

Diameter of Whatman paper = 6 mm, [1]=[2]=[3] = 10000 µg/mL
Note: 1. Curcusone B; 2. Stigmasterol; 3. Jatrophone; 4. Solvent ($CHCl_3$); 5. Control (teracyclin 30 µg/disc)

Table 3. Biological Activities of Terpenoids Against Murine Leukemia P-388 Cells

Compound	IC_{50} (µg/mL)
Curcusone B	0.57 (1.93 µM)
Stigmasterol	>100
Jatrophone	>100

Table 4. Value of MIC and MBC of Jatrophone

Bacteria	MIC (µg/mL)	MBC (µg/mL)
S. aureus	15.6	>500
B. anthracis	7.8	>500

Table 2 and Table 3 shows that curcusone B is better than jatrophone and stigmasterol to be developed as anticancer agent, meanwhile jatrophone has good potency as an antibiotics compared to curcusone B and stigmasterol. As an anticancer candidate, curcusone B has IC_{50} = 0.57 µg/mL (1.93 µM) or is considered into a very active category. Referring to standard of NCI USA, there are 3 levels of the cytotoxic properties of a compound to the murine leukemia P-388 cells, which is very active (IC_{50} 0.0-2.0 µg/mL), active (IC_{50} 2.0-4.0 µg/mL), and inactive (IC_{50} >4.0 µg/mL). Based on the above criteria, curcusone B is a highly active compound. This information can be a reference for evaluating cytotoxic property of curcusone B against other cancer cell lines, besides KB cells, Hep3B cells and MCF-7 cells [20], K562 cells and H1299 cells [21].

The potencies of curcusone B and jatrophone as antibacterial and antifungi are consistent with the results of activity testing of crude extract of *J. curcas* and *J. gossyfipolia* against bacteria and fungi [3,6,10]. As antibacterial and antifungal candidate, jatrophone is better than curcusone B (Table 2). Jatrophone was very active toward *S. aureus* (bacteria) and *A. niger* (fungi). Further study on jatrophone as new potential antibiotics was carried out on bacterial pathogens, *S. aureus* and *Bacilus anthracis* (Table 4). MIC (Minimum inhibitory Concentration) and MBC (minimum bactericidal Concentration) value indicated that jatrophone has bacteriostatis properties.

4. Conclusion

Three terpenoid compounds have been isolated from two stem bark of *jatropha* plants, which are curcusone B, jatrophone (diterpena) and stigmasterol (triterpena). The structure of all compounds was determined by comparing ^{1}H and ^{13}C NMR data of the compounds with literatures. Curcusone B was very active toward murine leukemia P-388 cells. So, this compound can be developed as an anticancer agent. Jatrophone indicated good potency for an antibiotic drug especially against

S. aureus. This compound has bacteriostatis properties toward *S. aureus*.

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