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Research Article

CYTOTOXIC POTENCY OF DITERPENES FROM *JATROPHA* PLANTS

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ABSTRACT

Objective: *Jatropha* plants produced various secondary metabolites which have interesting biological activities. This project will study isolation, structure elucidations and cytotoxic evaluation of diterpenes from *J. gossypifolia* and *J. curcas.*

Methodology: Isolation of the compound was carried out by using chromatography method i.e vacuum liquid chromatography (vlc) and radial chromatography (rc) with silica gel as an adsorbent and various solvents as eluen. The compound structures were evaluated by spectroscopic data (FTIR, UV and NMR data) and then compared with the same data from references. The cytotoxic properties of these compounds were evaluated towards HeLa (*cervical cancer*) and WiDr (*colon adenocarcinoma*) cell lines by using MTT assays.

Results: Three diterpenes, named jatrophone (**1**), curcusone B (**2**) and jatropholone A (**3**) have been isolated from *Jatropha* plants. Jatrophone (1) was isolated from stem Barks of *J. gossypifolia*, and curcusone B (**2**) and jatropholone A (**3**) were purified from stem Barks of *J. curcas*. Cytotoxic potency of the diterpenes were indicated by IC_{50} value of the compounds. Value of IC_{50} of jatrophone, curcusone B, jatropholone A and doxorubicicine (standard) toward WiDr cell lines are 8.97; 18.24; 15.20; and 9.00 µM, respectively. Meanwhile, IC_{50} of jatrophone, curcusone B, jatropholone A and tamoxifene (standard) toward HeLa cell lines are 5.13; 19.60; 36.15; and 7.80 µM, respectively.

Conclusions: Jatrophone had better potency as anticancer than curcusone B and jatropholone A. Activity of jatrophone against HeLa and WiDr cell lines was better than anticancer standards (tamoxifen and doxorubicine).

Keywords: Jatrophone, Curcusone B, Jatropholone A, HeLa and WiDr cell lines, MTT {3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide} assay.

INTRODUCTION

Two *Jatropha* plants, *J. 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 are 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, the grain`s crop is used to treat wounds and skin disorders [3], and in Comoro Island (Africa), leaves of this plant are used for a malarial drug [4]. Moreover, stem bark and root extracts of the plant 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.*) [5]. Extracts combination of *J. curcas* tissues; stem bark-root, stem bark-leaf, root-leaf, and stem bark-leaf-root are active extracts towards *Salmonella thypi* and *Escherichia coli* [6]. Extract of *J. gossypyfolia* is also employed as antibiotic and anti-fertility in human health. In India, the plant extract is used as toxic agent 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,* and *Candida albicans* [8]. Additionally, root of *J. curcas* proved as an anti inflammatory agent and diarrhea [9]. The leaves are toxic against the larva of *Culex quinquefasciatus* and the resin has potency as a coagulant [10]. For cytotoxic activities, extracts of *J. curcas* and *J. gossypyfolia* showed a significant antiproliferative activity towards HeLa cell lines [11].

Phytochemical study of *J. curcas* has successfully been identified for some compounds such as dinorditerpene [12]; and curcusone A-D from the roots [13]; jatrophalactone, jatrophalone, and jatrophadiketone [14]. Meanwhile, phytochemical study of *J. gossypifolia* has been successfully been identified jatrophone [15] and clemiscosin A [16]. In continuing of our study on biological activities of terpenoides of *Jatropha* plants, three diterpene compounds have been isolated and elucidated from *Jatropha* i.e. curcusone B and jatropholone A from *J. curcas*, and jatrophone from *J. gossypifolia.* Those compounds have interesting cytotoxic potency, showing that curcusone B is an active compound toward cell lines K562 and H1299 [17] and jatropholone A has antiproliferative activity against five fibroblasts CCL-171, AGS CRL-1739, lung HTB-58, bladder HTB-1 and leukemia CCL-240 at >100 lM. Reportedly, jatrophone has cytotoxic potency toward P-388 lymphocytic leukemia, KB cell lines, the P-388 lymphocytic leukemia and Eagle'scarcinoma of the nasopharynx test system, AGS and lung fibroblast, anti-proliferative effects against fibroblasts CCL-171, AGS CRL-1739, lung HTB-58, bladder HTB-1, and leukemia CCL-240 [18]. However, cytotoxic potency of curcusone B, jatropholone A and jatrophone toward HeLa (*cervical cancer cell line*) and WiDr (*colon adenocarcinoma cell line*), have not been known. This article is purposed to explain isolation of those compounds and their cytotoxic potency to the HeLa and WiDr cell lines.

MATERIAL AND METHODS

General

Isolation was conducted at Haluoleo University by using vacuum liquids chromatography methods (VLC). VLC methods was equipped with Merck Si-gel 60 GF254, and TLC analysis on pre-coated Si-gel plates with Merck Kieselgel 60 F254, 0.25 mm. UV spectra was measured using Cary Varian 100 conc. and IR spectra using Perkin-Elmer Spectrum One FT-IR Spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded with a JEOL ECP 500 spectrometer, operating at 500 MHz (1H) and 125 MHz (13C), worked at LIPI Serpong.

Plant Material

Samples 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 in Herbarium Bogoriense, Bogor Indonesia, and a voucher specimen was deposited at the herbarium. Cancer cell lines including cell lines HeLa (*cervical cancer cell line*) and WiDr (*colon adenocarcinoma cell line*) were determined at Pharmacology Laboratory Faculty of Pharmacy Hasanuddin University Makassar Indonesia.

Isolation

Isolation of compounds from stem Barks of *J. gossypifolia.*

Powder of stem bark of *J. gossypifolia* (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. F21 (1.2 g), F22 (3.0 g), F23 (4.8 g), F24 (2.2 g) and F25 (5.1 g). Purification of F23 resulted a white crystal (**1)** (33 mg).

Isolation of compounds from stem Barks of *J. curcas*.

The similiar method has been used for the isolation of jatrophone (**1**) from stem barks of *J. gossypifolia*, from the powder of stem barks of *J. curcas* (1.0 kg) has been isolated a yellow crystal compound (**2**) (300 mg) and a white crystal (**3)** (12 mg).

Structure determination of pure compounds

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

*Compound **1**.* A white crystal compound, m.p. 152-153 ^{o}C. Spectra of FTIR (KBr) ῡmaks (cm-1) 3283 (OH), 2961, 2929 (C*sp3*-H), 1690, 1654 (C=O), 1619 (C=C) and 1292 (C-O). Spectra of ^{1}H NMR (CD3OD, 500 MHz) δH (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 (CD3OD, 500 MHz) δc (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).

*Compound **2.*** A yellow crystal compound, melting point (m.p.) of 128-129 oC, [α]D 20 -543^o (*c* 0.1 MeOH), UVVis (MeOH) λmaks (log ε) 201 (5.36), 257 nm (3.67). IR spectra (KBr) showed at ῡmaks (cm-1) 3076 (C*sp2*–H),2956 (C*sp3*–H), 2928 (C-C alkyl), 1711 (C=O ketone), 1657 (C=O ketone), and 1641, 1445 (C=C).

Spectra of 1H NMR (CDCl3, 500 MHz) δH (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 (CDCl3, 125 MHz) δC (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 3. A white crystal compound, melting point (m.p.) of 128-129 oC, [α]D 20 -543^o (*c* 0.1 MeOH), UVVis (MeOH) λmaks (log ε) 201 (5.36), 257 nm (3.67).

Spectra of ^{1}H NMR ($CDCl_3$, 500 MHz) δH (ppm) 0.81 (3H, *s*, H-19), 0.92-0.86 (1H, *s*, H-9), 0.92-0.86 (1H, *s*, H-11), 1.17 (3H, *d*, 1,25 Hz, H-16), 1.23 (3H, *s*, H-18), 1.57-1.55 (1H, *m*, H-1b), 1.79 (1H, *d*, *J* = 2.4 Hz, H-1a), 2.25 (3H, *s*, H-20), 2.60-2.47 (2H, *m*, H-7), 2.60-2.47 (2H, *m*, H-8), 3.35-3.32 (1H, *m*, H-2), 4.54 (1H, *s*, H-7a), 5.06 (1H, *s*, H-7b), and 7.83 (1H, *s*, H-14 OH).

Spectra of ^{13}C NMR ($CDCl_3$, 125 MHz) δC (ppm) 13.1 (C-20), 15.5 (C-19), 16.4 (C-16), 18.9 (C-10), 21.3 (C-8), 25.8 (C-9), 27.5 (C-11), 28.4 (C-18), 30.3 (C-1), 33.5 (C-7), 42.2 (C-2), 114.4 (C-17), 131.2 (C-15), 131.7 (C-13), 132.7 (C-5), 136.9 (C-12), 137.5 (C-4), 145.5 (C-4), 151.3 (C-14), and 206.4 (C-3).

Antiproliferative Activity Assay (MTT Assay)

MTT assay procedure was outlined by Soundararajan [19]. The antiproliferative activity assay of compounds were measured using MTT {3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide} assay (Sigma). The assay detects the reduction of MTT by mitochondrial dehydrogenase to blue formazan product, which reflects the normal function of mitochondria and cell viability. Exponentially growing cells were washed and seeded at $1x10^4$ cells/well for HeLa cell line (in 200 µl of growth medium) in 96 well microplates (Nunc, Roskilde Denmark). After 24 h incubation, a partial monolayer was formed then the media was removed and 200 µl of the medium containing the compound (iniatially dissolved in DMSO) were added and re-incubated for 48 h. Then 100 µl of the medium were aspirated and 15 µl of the MTT solution were added to the remaining medium (100 µl) in each well. After 4 h contact with the MTT solution, blue crystals were formed. One 100 µl of the stop solution were added and incubated further for 1 h.

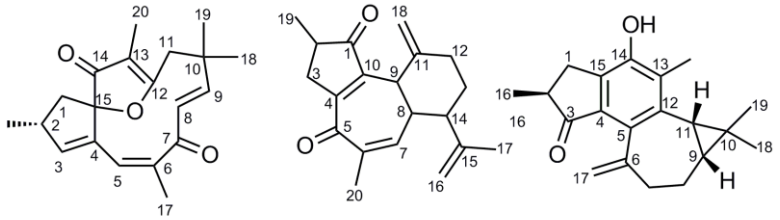
Reduced MTT was assayed at 550 nm using a microplate reader (Biorad). Control groups received the same amount of DMSO (0.1%). Untreated cells were used as a negative control, while cells were treated with tamoxifen as a positive control. Eight concentrations (125, 62.5, 31.25, 15.63, 7.81, 3.91 and 1.95 µg/ml) were prepared from each compound and tested against the HeLa cell line. IC_{50} values were calculated as the concentrations that show 50% inhibition of proliferation on tested cell line. Stock solutions of the compounds were dissolved in DMSO then diluted with the medium and sterilized using 0.2 µm membrane filters. The final dilution of the compound used for treating the cells contained not more than 0.1% (non-toxic concentration) DMSO. IC_{50} values were reported as the average of three replicates. The antiproliferative effect of tested compounds was determined by comparing the optical density of the treated cells against the optical density of the control. The cell viability (% of control) was calculated by the following equation:

$$\text{Cell viability \%} = \frac{(\text{Absorbace control}) - (\text{Absorbance sample})}{(\text{Absorbace control})} \times 100\%$$

The same procedure was applied to WiDr cell lines with doxorubicine as a standard compound.

RESULTS AND DISCUSSION

In this research, three diterpenes have been isolated from *Jatropha* plants, are known compounds. Structure elucidation of three compounds was determined by comparing the spectroscopic data of the isolated compounds with the relevant data which have been published (references).



Jatrophone (**1**) Curcusone B (**2**) Jatropholone A (**3**)

Table 1: Comparison of spectroscopy data of isolate (1) with jatrophone (1*) [15]

No. C	δ_C(1)	δ_C (1*)	No. H	δ_H(1)	δ_H(1*)
1	43,4	42,4	1a	2,20 (1H, *dd*, 7,95; 5,82)	2,17 (1H, *dd*, 13,7; 5,9)
			1b	1,79 (1H, *dd*, 7,95; 7,92)	1,88 (1H, *dd*, 13,7; 7,8)
2	39,7	38,3	2	2,98 (1H, *bm*, 2,45; 2,15)	3,00 (1H, *ddq*, 7,8; 7,0; 5,9)
3	124,5	123,7	3	5,76 (1H, *d*, 1,2)	5,83 (1H, *br s*)
4	138,8	137,0	-	-	-
5	148,3	147,0	5	5,77(1H, *d*, 1,2)	5,84 (1H, *br s*)
6	143,9	141,7	-	-	-
7	204,1	201,9	-	-	-
8	129,4	128,7	8	5,98 (1H, *d*, 15,9)	6,02 (1H, *d*, 16,4)
9	162,4	159,0	9	6,60 (1H, *d*, 16,5)	6,47 (1H, *d*, 16,4)
10	38,0	36,6	-	-	-
11	42,0	41,2	11a	3,04 (1H, *d*, 15,3)	2,89 (1H, *d*, 14,7)
			11b	2,50 (1H, *d*, 15,3)	2,43 (1H, *d*, 14,7)
12	187,0	183,2	-	-	-
13	113,9	112,4	-	-	-
14	206,3	203,8	-	-	-
15	101,5	99,7	-	-	-
16	19,7	18,9	16	1,10(3H, *d*, 6,75)	1,10 (3H, *d*, 7,0)
17	20,9	20,7	17	1,86 (3H, *d*, 1,25)	1,90 (3H, *s*)
18	30,5	30,4	18	1,26 (3H,*s*)	1,27 (3H, *s*)
19	27,2	26,9	19	1,37 (3H, *s*)	1,39 (3H, *s*)
20	6,0	6,0	20	1,72 (3H, *s*)	1,77 (3H, *s*)

Compound **1** was isolated as a white crystal compound, m.p. 152-153 ^{o}C. Spectra of FTIR showed that peak at 3283 cm^{-1} (hydroxyl). 2961, 2929 cm-1 (Csp3–H), and peaks at 1690 and 1654 cm^{-1} for two units of carbonyl (C=O), peaks at 1619 cm^{-1} for =C–C=O, and a peak at 1292 cm^{-1} for C-O ether. 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 *sp2* (C-3,4,5,6,8,9,12,13), and 2 carbon atoms *sp2* carbonyl, C=O (C-7, C-14). Meanwhile 1H NMR spectra showed 14 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 jatrophone (**1**). Confirmation of the structure was carried out by using NMR-2D (HMQC, HMBC, and H–H COSY) and by comparing the spectroscopic data (^{1}H and ^{13}C NMR) of isolate with spectroscopic data of library [13], see in Table 1. The data indicated to have highly suitable parameters between compound **1** and jatrophone. Consequently, It can be concluded that compound **1** is jatrophone. Structure determination of compounds **2** and **3** were carried out by using the similar prosedure as structure elucidation of compound **1** (jatrophone); so that compound **2** and compound **3** were believed as curcusone B [12] and jatropholone A [13], respectively.

Cytotoxic properties of jatrophone, curcusone B and jatropholone A were analyzed toward cell lines : HeLa (*cervical cancer cell line*) and WiDr (*colon adenocarcinoma cell line*) (Table 2).

Table 2: Cytotoxic properties of diterpenes towards selected cell lines

Cell Lines	IC_{50} (µM)			
	Jatrophone	Curcusone B	Jatropholone A	Standard
WiDr	8.97	18.24	15.2	Doxorubicine= 9.0
HeLa	5.13	19.6	36.15	Tamoxifen = 7.8

Based on the data at **Table 2** indicated that jatrophone has better potency as anticancer of colon and cervix than curcusone B and jatropholone A. Activity of jatrophone against WiDr and HeLa cell lines is better than anticancer standard (doxorubicine and tamoxifen). All compounds activities against HeLa cell lines supported activity data of extracts of *J. curcas* and *J. gossypyfolia* towards HeLa cell lines [11].

CONCLUSION

Three diterpenes have been isolated from *Jatropha* plants (*J. gossypifolia* and *J. curcas*), identified as jatrophone, curcusone B and jatropholone A. Cytotoxic potency of those compounds toward HeLa and WiDr cell lines indicated that jatrophone has better potency than curcusone B and jatropholone A. Specifically, jatrophone is more active than anticancer standard against HeLa and WiDr cell lines.

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