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Antiproliferative Activity of Curcusone B from *Jatropha Curcas* on Human Cancer Cell Lines

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Abstract: This study was designed to determine antiproliferative properties of curcusone B from the stem bark of *Jatropha curcas* on cell lines K562 (chronic myelogeneous leukemia), and H1299 (human non-small cell lung carcinoma). Cells were cultured in the presence of curcusone B at various concentrations and the percentage of cell viability was evaluated by trypan blue exclusion method and MTT assay. Curcusone B showed significant dose-dependent inhibition of cell proliferation of K562 and H1299 cell lines with an IC_{50} of 6 µg/mL and 15.0 µg/mL, respectively. The findings suggested that the curcusone B had strong antiproliferation which was dose dependent and may have potential as an anticancer agent.

Key words: *Jatropha curcas*, Curcusone B, Antiproliferative, cell culture.

INTRODUCTION

Cancer is a leading cause of death worldwide with increasing morbidity and mortality. It can affect any part of the body (Katzung, 2007). Cytotoxic chemotherapy has been one of the mainstays of cancer treatment for many years and is also said to be the most rapidly developing field of cancer treatment, with new drugs which include plant metabolites being constantly screened and tested (Kintzios and Barberaki, 2003). *J. curcas* (family Euphorbiaceae), also known as physic nut, purging nut or pig nut, is a multipurpose bush/small tree popular for both economic (Akbar *et al*., 2009a) and medicinal uses (Akbar *et al*., 2009b). It is originally from tropical America, but also distribute in many parts of the tropics and sub-tropics in Africa-Asia. As with many Jatropha species, *J. curcas* is used in the traditional folklore medicine to cure various ailments in Africa, Asia, and Latin America (Burkill, 1994). Leaves and roots are used as a remedy for cancer, as an abortifacient, antiseptic, diuretic, purgative, external application for the treatment of skin diseases, sores, rheumatism, piles and as an antidote for snake-bites. The nut of the plant has also been used for the treatment of burns, convulsions, fever and inflammation. Other uses from its oil include soap making, illumination, cosmetics, organic manure and most recently as a potential alternative to diesel (Akbar *et al*., 2009a; Kumar and Sharma, 2005; Osoniyi and Onajobi, 2003). Studies have revealed that the plant contains phytochemicals such as saponins, steroids, tannins, glycosides, alkaloids and flavonoids, and some of them exhibit significant biological activity (Akbar *et al*., 2009a; Akbar *et al*., 2009b). Curcusone B is one of the four curcusones isolated from the roots of *Jatropha curcas* and belongs to the crotophorbolane class of compounds (Naengchomnong *et al*., 1986). Curcusone B isolated from the stem barks of *J. curcas* (Ardiansyah *et al*., 2009) was reported to possess potential antimicrobial and anticancer activity. From the above traditional uses and later scientific findings suggested that *J. curcas* is a potential candidate as an antimicrobial and anticancer agent. To the best of our knowledge, there is no publication available on the possible antiproliferative activity of curcusones from *J.*

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curcas on human cancer cells. Therefore, the possibility to obtain such findings is high and may provide interesting information on the plant's activity as an anticancer.

Experimental:

Reagents:

RPMI 1640 medium, Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS) and Penicillin-Streptomycin were obtained from GIBCO/Invitrogen (Auckland, NZ); Thiazolyl Blue Tetrazolium Bromide (MTT) from Sigma-Aldrich (USA) and dimethyl sulphoxide (DMSO) from Merck (Germany).

Plant Material:

The stem bark of *J. curcas* was obtained from Collection and Development Centre for Traditional Medicine of South East Celebes Community, Arboretum Prof. Mahmud Hamundu, Haluoleo University. This plant had been identified by staff of Herbarium Bogoriense, Bogor. Ground-powder of *J. curcas* stembark (1.0 kg) was macerated with methanol (3 x 3L) for 3 days. Methanol extract was concentrated at low pressure and dark brown gum was obtained (100 g) as previously described (Ardiasyah *et al*., 2009). Using vacuum column chromatography, the extract was subsequently rechromatographed until 0.3 g of pure compound was yielded eventually. The compound was kept at 4 °C and dissolved in dimethyl sulphoxide DMSO to make a concentration of 10 mg/ml.

Cell culture:

K-562 cell line was obtained from the American Type Culture Collection (Rockville, MD) and was cultured in RPMI 1640. H1299 cell lines were a courtesy of Prof. Masa-Aki Ikeda of Tokyo Medical and Dental University and were cultured in DMEM medium. All media were supplemented with 10% (v/v) FBS, 100 mg/L of streptomycin and 100,000 U/L of penicillin.

Cell Proliferation Assay:

K-562 cell line. Columns of a 48-well microplate were seeded with 250 µl of cell suspension per well and serial dilutions of curcusone B were immediately added. Solvent controls were treated with 0.1% DMSO. Cells were then incubated for 48 h. A hemacytometer count for both control and treatment groups was done with trypan blue under light microscope. The antiproliferative rate for each concentration was determined according to the following equation.

$$\text{The \% cell viability} = \frac{\text{\% viability treated cells}}{\text{\% viability control cells}} \text{x } 100$$

H1299 Cell Lines:

Columns of 96-well microplates were seeded with 100 µl of cell suspension per well and incubated for 72 h in a CO_2 incubator at 37 °C in humidified 5% CO_2 atmosphere. Serial dilutions of curcusone B (50 µl) were then added into each of 96-well plates. The cells were then incubated for another 48 hrs. After incubation, the medium was removed and cells in each well were incubated with PBS contained 1 mg/mL MTT for 24 h at 37 in 5% CO_2 incubator. After this 100 µl of DMSO was added into each well to dissolve the insoluble formazan crystal. Plates were then incubated for 4 h at 37°C for complete solubilization. The level of colored formazan derivative was analysed on a microplate reader (Tecan) at a wavelength of 570 nm (Abe and Matsuki, 2000). The percentage viability of the cells was calculated using the following equation.

$$\text{The \% cell viability} = \frac{\text{Mean Abs sample}}{\text{Means Abs control}} \text{x } 100$$

Where; Abs. = Absorbance

Statistical Analysis:

The experiments were done in triplicates to generate statistically relevant data and the results were expressed as mean ± SD using the SPSS 15.0 software program, statistical analysis was done with Two Way Anova. $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effect of Curcusone B on the Proliferation of K-562 Human Leukemia Cell Line:

The relationship between the concentration of curcusone B and its cytotoxic effect on proliferation of K562 chronic leukemia cell line was investigated by trypan blue exclusion assay. Cells were treated with curcusone B at concentrations ranging from 3.13 to 100 ug/mL for 48 h and then the percentage of cell viability was analysed as described above. The mean % viability obtained at different compound concentrations is shown in Table 1. Values have been expressed as mean ± SD (n=3).

Table 1: % Viability of K562 cell line after treatment with different concentrations of Curcusone B.

Sample	% Viability at different concentrations					
	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25ug/mL	3.13 µg/mL
Curcusone B	1.30±1.84	3.35±1.48	13.95±1.06	30.63±0.88	49.90±2.26	65.20±0.42
DMSO	92.35±7.99	88.35±4.73	95.10±0.57	91.90±9.05	96.60±1.83	99.30±0.99

Curcusone B from the stem bark of *J.curcas* significantly inhibited the proliferation of K562cells ($P<0.001$), in a dose-dependent manner (Figure 1). The IC_{50} was observed at 6 µg/mL by graphical interpolation.

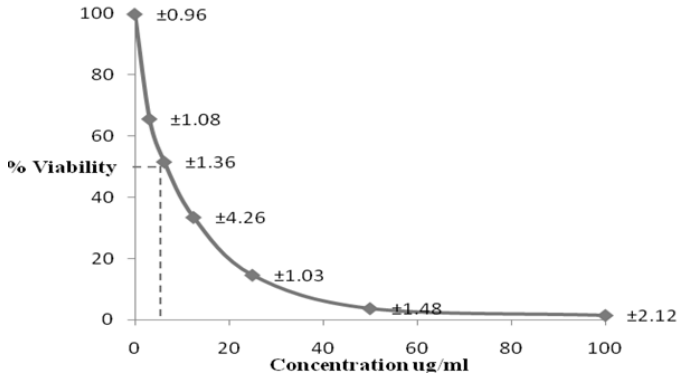

Fig. 1: Effect of curcusone B on the proliferation of K562 cell line. The percentage viability was determined with trypan blue exclusion assay. Data represents the means ± SD (*n*=3). IC_{50} was estimated at 6 µg/ml.

Effect of Curcusone B on the Proliferation of H1299 Human Non-small Cell Lung Cell Line:

The relationship between the concentration of curcusone B and its cytotoxic effect on proliferation of H1299 cell lines was investigated by MTT assay. Cells were treated with curcusone B at concentrations ranging from 3.13 to 100 µg/mL for 48 h and then the percentage of cell viability was analyzed as described before. The mean absorbance obtained after treatment of H1299 cell line with different compound concentrations is shown in Table 2. Values have been expressed as mean ± SD (n=3).

Table 2: Mean absorbance obtained after treatment of H1299 cell line with different concentrations of Curcusone B

Sample	Mean absorbance at different concentrations					
	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.13 µg/mL
Curcusone B	0.187±0.006	0.439±0.034	0.514±0.013	0.842±0.038	0.971±0.013	1.232±0.175
DMSO	0.507±0.031	1.211±0.041	1.308±0.074	1.563±0.033	1.501±0.060	1.899±0.153

Curcusone B showed significant inhibition ($p= 0.018$) of the proliferation of H1299 cells in a dose-dependent manner (Figure 2). The IC_{50} was observed at 15µg/ml by graphical interpolation.

Although *J. curcas* has long been used as traditional medicine, few scientific studies in the field of cancer therapy are available. Recent in vitro studies have shown that many constituents from *J. curcas* have a wide range of biological actions including antibacterial, antifungal, antiparasitic, haemostatic and strong anti HIV-induced cytopathic effects with low cytotoxicity (Akbar *et al*., 2009a; Osoniyi and Onajobi, 2003). Studies have revealed that *J. curcas* is a source of saponins, steroids, tannins, glycosides, alkaloids, and flavonoids (Akbar *et al*., 2009b) and some of them have relevant biological activities.

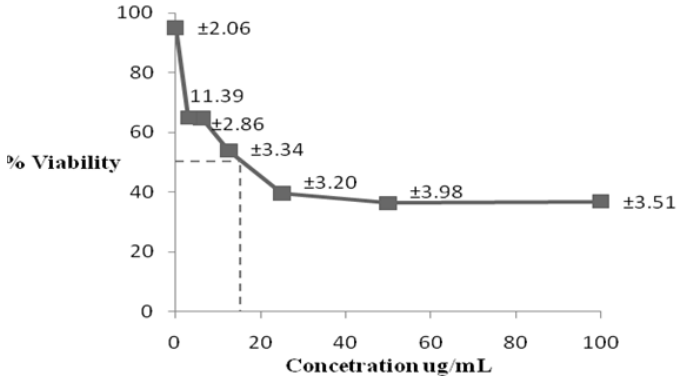

Fig. 2: Effect of curcusone B on the proliferation of H1299 cell line. The percentage viability was determined with MTT assay. Data represent the means ± S.D. (n =3). IC_{50} was estimated at 15 µg/ml.

In this study, the antiproliferative activity by curcusone B from the stem bark of *J. curcas* on human cancer cell lines was investigated. It was found that curcusone B significantly inhibited the proliferation of K-562 and H1299 cells after an incubation period of 48h. The antiproliferative effect was evaluated by trypan blue exclusion and MTT assays respectively.

The results presented have shown a concentration dependant decrease in the percentage of cell viability and, at a concentration of 3.13-100 µg/ml, curcusone B was sufficient to inhibit the cell proliferation of K562 and H1299. Thus curcusone B displayed strong antiproliferative activity on both human chronic myelogenous leukemia and non-small cell lung carcinoma cells with an IC_{50} of 6.1µg/mL and 15µg/mL, respectively.

In conclusion, the results demonstrated that curcusone B from the stem bark of *J. curcas* has a significant antiproliferative effect. This is in line with findings from previous studies that have revealed the potential of *J. curcas* in possessing relevant antimicrobial and anticancer effects. One study (Zeng *et al*, 2004) determined the in vitro antibiotic effect of an alcohol extract from jatropha leaf on *Escherichia coli* and *Staphylococcus aureus.* The extract inhibited *E. coli* and *S. aureus*, and the activity against *E. coli* was better than that against *S. aureus.* Curcin, from seeds of *J. curcas*, was found to be a ribosome inactivating protein (RIP) in a study which revealed the functional mechanism of curcin at molecular level for the first time. It was discovered that curcin had an antitumor effect with mechanisms of action related to N-glycosidase activity (Lin *et al*., 2002; Lin and Chen, 2003; Lin *et al*., 2003). The present study reveals for the first time that curcusone B from the stem bark of *J. curcas* exhibited a significant antiproliferative effect on K562 and H1299 cell lines.

These probable properties of *J. curcas* provide scope of further detailed investigation of purified compounds from this plant because of their potential as antineoplastic agents. Moreover, other biological activities on different cell lines and microbial organisms which may be correlated to traditional treatments of *J. curcas* should be invetigated as well as hematological disorders and chronic infetcions.

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REFERENCES

Abe, K. and N. Matsuki, 2000. Measurement of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) reduction activity and lactate dehydrogenase release using MTT. Neuroscience Research, 38: 325-329.

Akbar, E., Z. Yaakob, S.K. Kamarudin, M. Ismail and J. Salimon, 2009a. Characteristic and Composition of *Jatropha Curcus* Oil Seed from Malaysia and its Potential as Biodiesel Feedstock. European Journal of Scientific Research, 29(3): 396-403.

Akbar, E., O.O. Igbinosa, E.O. Igbinosa and O.A. Aiyegoro, 2009b. Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcus* (Linn). African Journal of Pharmacy and Pharmacology, 3(2): 058-062.

Ardiyansyah, I. Sahidin and M. Taher, 2009. Profile of Cucusone B from stembark of *Jatopha curcus* as an antimicrobial and anticancer agent. Paper presented at the Malaysian National Product International Seminar (MNPIS) 2009. Kuantan, Pahang.

Burkill, H.M., 1994. The useful plants of west Tropical Africa (Familiies E-J), Royal Botanical Gardens Kew, London UK. pp: 90-94.

Katzung, B.G., 2007. *Basic and Clinical Pharmacology* (10th Edition). USA: McGraw Hill, pp: 878-879.

Kintzios, S.E. and M.G. Barberaki, 2003. *Plants that Fight Cancer*. USA: CRC press, pp: 4-5.

Kumar, N. and P.B. Sharma, 2005. *Jatropha curcas*-Asustainable source for production of biodiesel. Journal of Scientifc and Industrial Research, 64: 883-889.

Lin, J., F. Yan, L. Tang and F. Chen, 2002. Isolation, purification and functional investigation on the N-Glycol sidase activity of curcin from the seeds of *Jatropha curcas*. High Technology Letters, 11: 36-40.

Lin, J. and Y. Chen, 2003. Cloning and expression of curcin, a ribosome-inactivating protein from the seeds of *Jatropha curcas*. Acta Botanica Sinica, 45(7): 858-863.

Lin, J., F. Yan, L. Tang and F. Chen, 2003. Antitumor effects of curcin from *Jatropha curcas* L. Acta Pharmacologica Sinica, 24(3): 241-246.

Naengchomnong, W., Y. Thebtaranonth, P. Wiriyachitra, K.T. Okamoto and J. Clardy, 1986. Isolation And Structure Determination of Four Novel Diterpenes. Tetrahedron Letter, 27(22): 2439-2442.

Osoniyi, O. and F. Onajobi, 2003. Coagulant and anticoagulant activities in *Jatropha curcas* latex. Journal of Ethnopharmacology, 89(1): 101-105.

Zeng, L.H., F. Yan and F.Chen, 2004. In vitro bacteriostasis of *Jatropha curcas* L.extract against chicken *Escherichia coli* and *Staphlococcus aureus*. Chinese Poultry, 8(1): 35-37.