Journal of Medicinal Plants Research Vol. 5(1), pp. 49-57, 4 January, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Antioxidant, anti-inflammatory and anticancer activities of methanolic extracts from *Jatropha curcas* Linn.

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Accepted 25 October, 2010

A comprehensive study on the phytochemical contents and biological activities of the methanolic extract from different parts of *Jatropha curcas* Linn. was conducted. The extracts of different plant parts contained various levels of phenolics, flavonoids and saponins. Latex and leaf extracts showed the highest antioxidant activity. Root and latex extracts inhibited the inducible nitric oxide synthase in macrophages RAW 264.7, comparable to L-Nitro-Arginine Methyl Ester (L-NAME), indicating appreciable anti-inflammatory activities. Cytotoxicity assay results indicated the anticancer therapeutic property of the root extract against human colon adenocarcinoma (HT-29) cell line but its cytotoxic effect on human hepatocyte (Chang cell) was high.

Key words: *Jatropha curcas* Linn., phytochemicals, antioxidant, anti-inflammatory, cytotoxicity.

INTRODUCTION

Jatropha curcas Linn. is a multipurpose plant in the *Euphorbiaceae* family. The oil from *J. curcas* seed is regarded as a potential fuel substitute since 40 to 50% of *J. curcas* seed oil can replace diesel without any engine modification (Pramanik, 2003). Phytochemical analyses have shown that different parts of *J. curcas* plant contain phenolic, flavonoid, saponin and alkaloid compounds (Thomas et al., 2008). Currently, plant derived-bioactive compounds have received considerable attention due to their therapeutic potential as antimicrobial, anti-inflammatory, anticancer and antioxidant activities (Rathee et al., 2009). Several studies have shown the antioxidant and anti-inflammatory properties of flavonoids and saponins present in various plant extracts (Vanacker et al., 1995; Seyoum et al., 2006; Sur et al., 2001; Rathee et al., 2009).

Recently, Ismail et al. (2010) and Kazlowska et al. (2010) reported similar biological activities attributed to the phenolic compounds present in cantaloupe (*cucumis melo*) and *Porphyra dentata*, respectively. Triterpene saponins also showed cytotoxic activity (Tundis et al., 2009). Applications of different varieties of *J. curcas* in traditional medicine have been reported by Ross (1999) and Kumar and Sharma (2008). However, information regarding the bioactive compounds and the therapeutic activities of the locally grown *J. curcas* is still lacking. Therefore, this research was conducted to evaluate the phytochemical contents, antioxidant, anti-inflammatory and cytotoxicity properties of methanolic extracts of leaf, stem bark, root and latex of the local *J. curcas* plant.

EXPERIMENTAL

Plant materials and crude extracts preparation

The *J. curcas* fresh whole plant (*Euphorbiaceae*) was collected from the farm of the Faculty of Agriculture, Universiti Putra Malaysia and identified by Mr. Shamsul Khamis from the Institute of Bioscience, Universiti Putra Malaysia. A voucher specimen (SK1764/2010) was

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deposited in the Phytomedicinal Herbarium, Institute of Biosciences, Universiti Putra Malaysia, Serdang, Selangor. The fresh *J. curca*s leaves, stem bark, root and latex were collected and freeze-dried to achieve constant weight followed by grinding. Five gram sample was soaked in 50 ml 80% (v/v) aqueous methanol and stirred overnight at ambient temperature. Each sample was centrifuged at 3000×*g* for 10 min and the supernatant was kept at -20°C. The extraction was repeated twice. The supernatants were pooled and evaporated by using a vacuum rotary evaporator (Buchii, Switzerland) to obtain the dry crude extract. The extracts were re-dissolved in methanol for antioxidant or in dimethyl sulfoxide for anti-inflammatory and cytotoxicity assays.

Total phenolic and flavonoid compounds

The total phenolic and flavonoid compounds in the extracts were determined according to Ismail et al. (2010). Results of total phenolic and flavonoid compounds were expressed as mg of gallic acid and rutin equivalents per gram dry matter (DM) respectively.

Total saponins

Total saponins of extracts were determined according to Makkar et al. (1999) and results were expressed as mg diosgenin equivalent/g DM.

Antioxidant activity

Free radical scavenging activity

Radical scavenging activity of extracts against stable DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) was determined spectrophotometrically as described by Ismail et al. (2010). The experiment was carried out in triplicate and results were reported as percentage of inhibition and IC_{50} (required concentration to scavenge 50% of DPPH radicals). Ascorbic acid and quercetin were used as standard antioxidants.

Nitric oxide scavenging activity

The NO-scavenging activity of extracts was determined according to Tsai et al. (2007). The activity was expressed as percentage of inhibition and IC_{50} (extract concentration to quench 50% of the NO radicals released by sodium nitroprusside).

Anti-inflammatory activity

The murine monocytic macrophage RAW 264.7 cell line (European Cell Culture Collection, CAMR, UK) was cultured in Dulbecco's Modified Eagle Media (DMEM) (2mM L-glutamine, 45 g/l glucose, 1 mM sodium pyruvate) with 10% fetal bovine serum (FBS). The cells were cultured at 37°C with 5% CO_2 and were subcultured twice a week. The cells were seeded in 96-well tissue culture plates (1 x 10^6 cells/ml) and incubated for 2 h at 37°C with 5% CO_2. Then, 100 µl of test extract in DMSO was then added and serially diluted to give a final concentration of 200 µg/ml in 0.1 % DMSO. Cells were then stimulated with 200 U/ml of recombinant mouse interferon-gamma (IFN-γ) and 10 µg/ml *Escherichia coli* lipopolysaccharide (LPS) and incubated at 37°C for another 17 h. The presence of nitrite was determined in cell culture medium by Griess reagent and cell viability was detected by using MTT cytotoxicity assay as described by Ahmad et al. (2005). N-nitro-l-arginine-methyl ester (L-NAME) was used as iNOS inhibitor (control) at a concentration of 250 µM.

Cytotoxicity

Human hepatocytes (Chang liver cells) and human colon adenocarcinoma (HT-29) cell lines obtained from the American Type Culture Collection (ATCC) were used in this study. Cells were grown at 37°C in humidified 5% CO_2 and 95% air atmosphere in Dulbecco's Modified Eagle Media (DMEM) (2mM L-glutamine, 45 g/l glucose, 1 mM sodium pyruvate, 2g /l sodium bicarbonate and 10% fetal bovine serum). Monolayers of the cells (5×10^3/100 µl) were grown in 96-well microtitre plates and exposed to two fold serial dilution of the extracts from 200 µg to 3.1 µg/100 µl. After 3 days incubation at 37°C, the cytotoxicity of extracts were determined by using MTT assay according to Ahmad et al. (2005). Tamoxifen, which is known as an anticancer drug was used as a positive control in the present study.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) and treatments mean were compared to control by using Dunnett's Multiple Comparison Test. GraphPad Prism 5 software (GraphPad Software Inc., San Diego, CA) was used for all the statistical analyses as well as IC_{50} value calculation.

RESULTS AND DISCUSSION

Phytochemical content

Table 1 shows the total phenolic, flavonoid and saponin contents of various parts of *J. curcas* plant. Leaf and latex contained higher phenolic compounds than other parts with values of 38.8 and 26.0 mg gallic acid equivalent/g DM, respectively. Root and stem bark were low in phenolics, containing 8.0 and 6.1 mg gallic acid equivalent/g DM, respectively. As indicated in Table 1, the highest flavonoid content was found in the latex with a value of 16.3 mg rutin equivalent/g DM followed by leaf, root and stem bark with values of 1.4, 1.06 and 0.09, respectively. In general, total saponin content was high in all plant parts with latex showing the highest saponin content (96.7 mg diosgenin equivalents/g DM) followed by leaf, root/stem bark with values of 66.2 and 58.0 mg diosgenin equivalents/g DM respectively. The results obtained were in agreement with previous researchers who reported the presence of phenolic, flavonoid and saponin compounds in *J. curcas* leaf, root and stem (El Diwani et al., 2009; Manpong et al., 2009). The presence of saponins (2.6 to 3.4% diosgenin equivalent) in the seed of *J. curcas* has been reported by Makkar and Becker (2009).

Antioxidant activity

Free radical scavenging activity

The free radical scavenging activity of methanolic

Table 1. Total phenolic, flavonoid and saponin contents of different parts of *J. curcas* plant.

Sample	Total phenolic[a] (mg/g DM)	Total flavonoid[b] (mg/g DM)	Total saponin[c] (mg/g DM)
Leaf	38.8±2.14	1.4±0.12	66.2±0.32
Stem bark	6.1±0.16	0.1±0.01	58.0±0.96
Root	8.0±0.15	1.1±0.10	58.0±1.08
Latex	26.0±0.91	16.3±0.30	96.7±0.73

[c] Diosgenin equivalent. [a] Gallic acid equivalent. [b] Rutin equivalent.

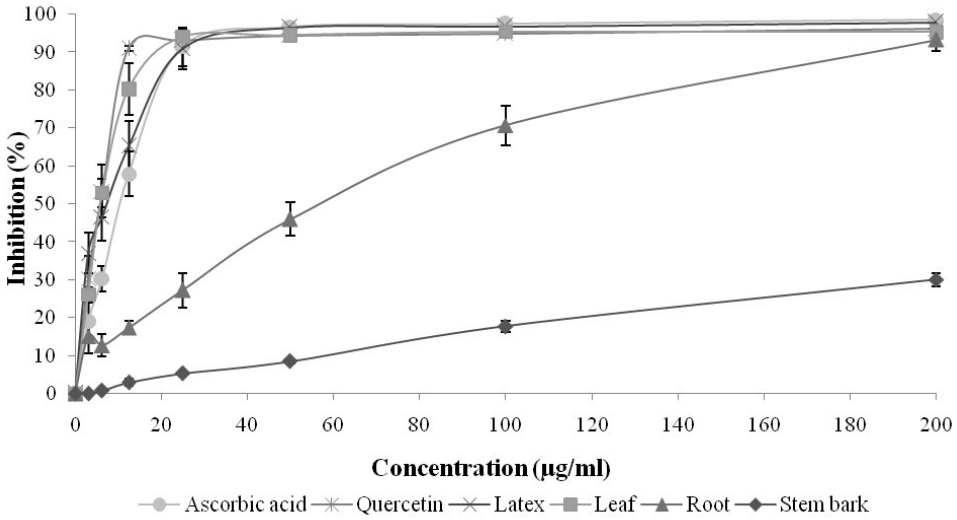

Figure 1. Free radical scavenging activity of extracts from various parts of *J. curcas*.

Table 2. The IC_{50} values of extracts and standards on free radical and nitric oxide scavenging activities.

Sample	IC_{50} (µg/ml)	
	Free radical scavenging activity	Nitric oxide scavenging activity
Stem bark	> 200	> 200
Leaf	6.8±2.71	93.5±4.03
Root	57.9±8.13	281.0±3.5
Latex	5.9±3.26	29.7±0.95
Quercetin	4.2±1.86	19.4±0.65
Ascorbic acid	10.6±1.75	40.0±1.17

extracts and controls are shown in Figure 1. Latex and leaf extracts showed similar scavenging activity when compared to quercetin and vitamin C. The IC_{50} values for DPPH scavenging activity for latex and leaf extracts, quercetin and vitamin C were 6.8, 5.9, 4.2 and 10.6 µg/ml (Table 2). The root and stem bark showed moderate and weak activities with IC_{50} values of 57.9 and >200 µg/ml, respectively.

The DPPH method has been recommended as an easy and accurate technique to measure the antioxidant activity of fruit and vegetable juices or extracts. The results are highly reproducible and comparable to other free radical scavenging method such as ABTS (Sanchez-Moreno, 2002).

Nitric oxide (NO) scavenging activity

Figure 2 shows the NO scavenging activity of samples tested. All samples exhibited NO scavenging activity in a dose-dependent manner. The corresponding IC_{50} values for NO scavenging activity are presented in Table 2. Latex and leaf extracts showed IC_{50} values below 200 µg/ml, indicating good NO-scavenging activity whereas

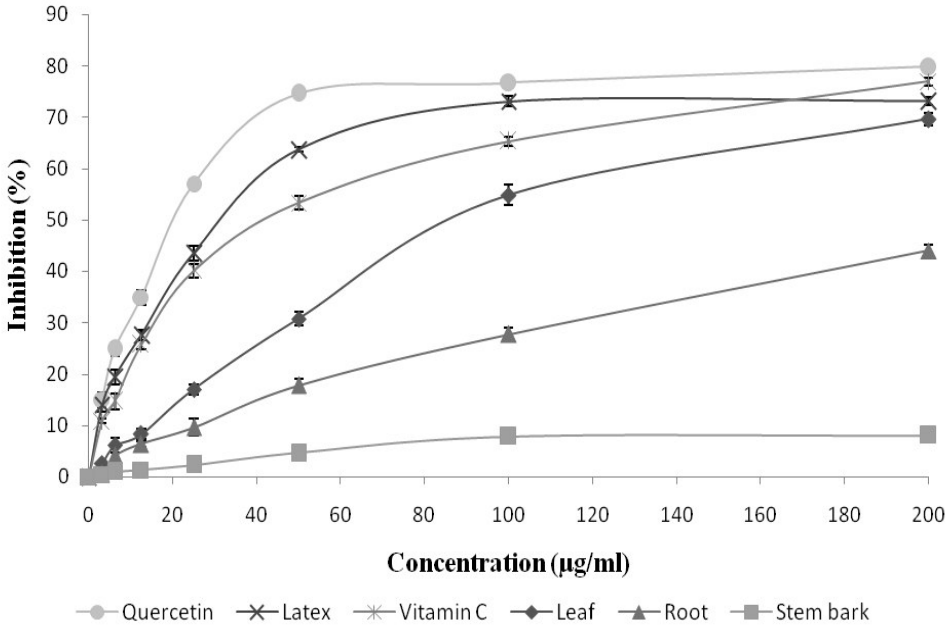

Figure 2. Nitric oxide scavenging activity of extracts from various parts of *J. curcas.*

root with the IC_{50} value between 200 to 400 µg/ml indicated moderate activity, while stem bark was observed to be a poor NO-scavenger. All the NO-scavenging values were categorized according to Tsai et al. (2007). The results of NO scavenging activity demonstrated that latex and leaf were good scavengers with IC_{50} values of 29.7 and 93.5 µg/ml, respectively.

Phenolic and flavonoid compounds which occur ubiquitously in plants are known to possess a variety of biological activities. These compounds showed linear correlations with free radical scavenging, NO scavenging and total antioxidant activities (Miliauskas et al., 2004; Seyoum et al., 2006; Vanacker et al., 1995; Yen et al., 2001). In addition, the contribution of saponins in antioxidant activity has been reported in several studies (Rodrigues et al., 2005; Sur et al., 2001). The free radical and NO scavenging activity of samples correlated well with the levels of phenolic, flavonoid and saponin present in the latex and leaf (Table 1).

Anti-inflammatory activity

The crude extracts of different parts of *J. curcas* were analyzed for their inhibitory effects on NO production from macrophages RAW 264.7 cells, induced by LPS and IFN-γ. The activity profiles of the extracts in terms of percentage of NO inhibition were categorized according to Kim et al. (1998). Figures 3 and 4 show the NO inhibition and cell viability of L-NAME, leaf and stem bark methanolic extracts. L-NAME inhibited NO production by 92.3% at the concentration of 250 µM while the cell viability was 98.3%. Only at 200 µg/ml, the NO inhibition values of leaf (80.8%) and stem bark (86.0%) methanolic extracts did not significantly differ from that of L-NAME. Even though this inhibition activity could be considered as strong iNOS inhibitor according to Kim et al. (1998), the reduction in cell viability (84.2 and 86.1 %) of raw 264.7 cell indicate the presence of toxic compounds in the extract.

It was noted that, latex extract at concentrations between 3.1 to 200 µg/ml (Figure 5) were not toxic to the raw 264.7 cell. However, the percentage of NO inhibition increased in a dose-dependent manner, but at 50, 100 and 200 µg/ml the NO inhibition values, were not significantly different from that of L-NAME. At 200 µg/ml, the value of NO inhibition was 93.9 %. These results indicate the strong ability of latex extract to inhibit the iNOS while maintaining cell viability comparable to L-NAME.

Figure 6 represents the NO inhibition and cell viability of raw 264.7 cells by root methanolic extract. Interestingly, all concentrations ranging from 3.1 to 200 µg/ml inhibited NO production with values from 93.6 to 95.8% similar to L-NAME which indicated strong iNOS inhibitor based on Kim et al. (1998) classification. However, concentrations from 6.2 to 200 µg/ml were found to be toxic to the raw 264.7 cell.

The results obtained suggest that leaf, stem bark, root and latex are strong iNOS inhibitor contributing to their anti-inflammatory effects. Similar findings on the anti-inflammatory effect of extracts from different parts of *J. curcas* plant has been reported. Mujumdar and Misar (2004) observed the anti-inflammatory activity of topical

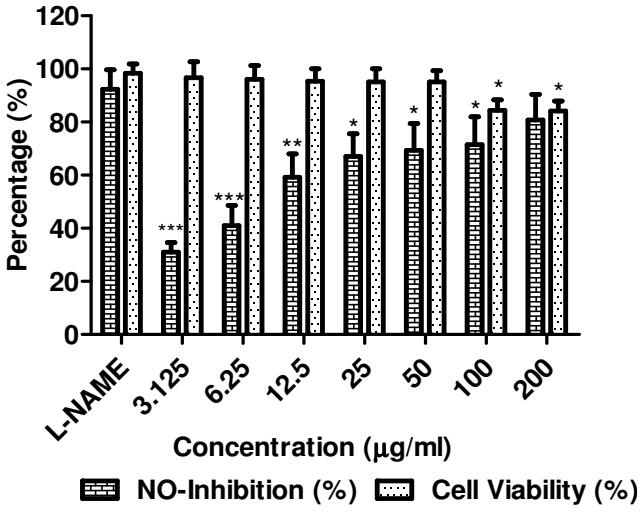

Figure 3. Percentage values of NO inhibition and cell viability of *J. curcas* leaf extract. All values represent mean ± standard deviation, ***P < 0.001, **P < 0.01 and *P < 0.05 indicate significant difference compared to the control (L-NAME).

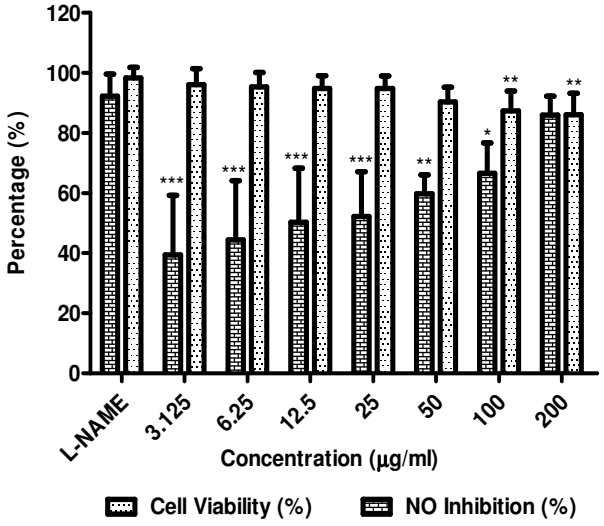

Figure 4. Percentage values of NO inhibition and cell viability of *J. curcas* stem bark extract. All values represent mean ± standard deviation, **P < 0.01 and *P < 0.05 indicate significant difference compared to the control (L-NAME).

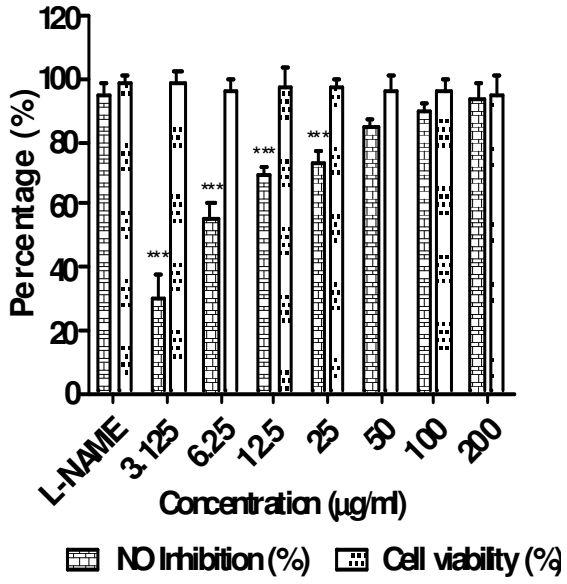

Figure 5. Percentage values of NO inhibition and cell viability of *J. curcas* latex extract. All values represent mean ± standard deviation, ***P < 0.001 indicate significant difference compared to the control (L-NAME).

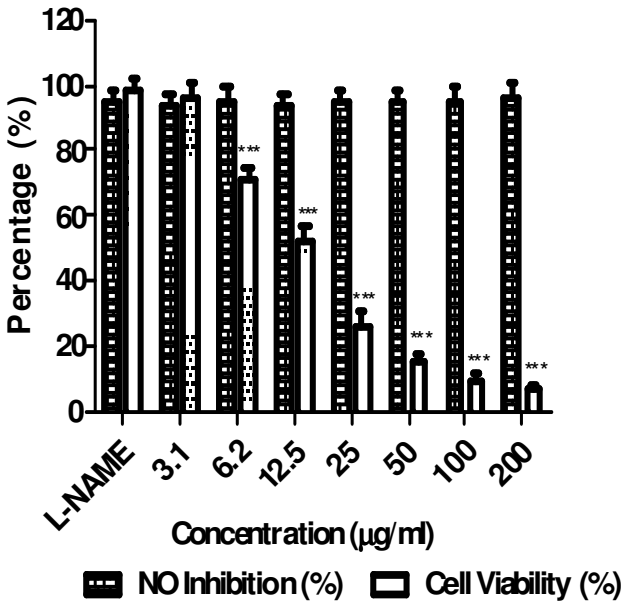

Figure 6. Percentage values of NO inhibition of *J. curcas* root extract. All values represent mean ± standard deviation, ***P < 0.001 indicates significant difference compared to the control (L-NAME).

application of *J. curcas* root powder paste, on TPA-induced ear inflammation in albino mice. Similarly, Uche and Aprioku (2008) reported the inhibition activity of *J. curcas* leaf extract, on the egg albumin induced inflammation in Wister albino rats.

Among plant parts, latex seemed to be promising as an anti-inflammatory agent, as it strongly inhibited iNOS and at the same time was non-toxic to raw 264.7 cell. Latex contained the highest amount of flavonoid and saponin compounds when compared to other plant parts and appreciable level of phenolics (Table 1). The mechanism of phenolic compounds in antioxidant activity and their

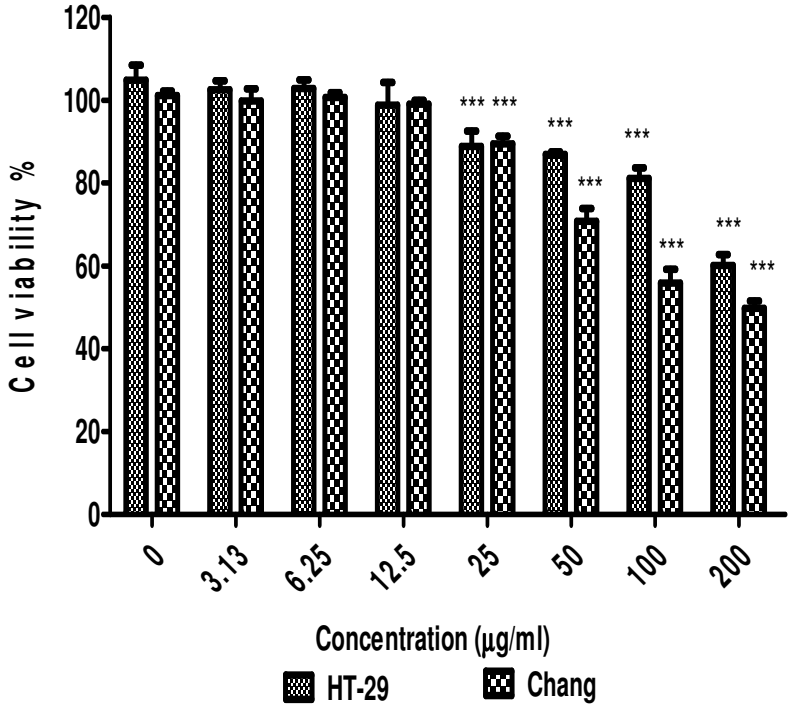

Figure 7. Effect of crude methanolic extract of *J. curcas* leaf on HT-29 and Chang liver cell viability. All values represent the mean ± S.D from three independent experiments. ***P < 0.001 indicates significant difference compared to the untreated control group.

ability to act as free radical and NO scavengers, leading to the formation of phenoxyl radicals have been described by Sumanont et al. (2004). Recently, Kazlowska et al. (2010) suggested that the inhibition of iNOS in the RAW 264.7 cell, is due to the suppressing action of flavonoids.

Furthermore, isolated saponins from roots of *Physospermum verticillatum*, exerted significant inhibition of NO production in LPS induced RAW 264.7 macrophages (Tundis et al., 2009). These findings augur well with the results of the present study which showed that latex extract with the best anti-inflammation activity, contained the highest amount of saponin. However, the presence of other phytochemical compounds could also contribute to the anti-inflammation effect.

Cytotoxicity activity

The results of cytotoxicity activity of leaf and stem bark extracts are shown in Figures 7 and 8, respectively. Increase in extracts concentration of up to 200 µg/ml, could reduce the cell viabilities significantly ($P < 0.001$) in a dose-dependent manner in both cell lines. The IC_{50} values of extracts used in this study are presented in Table 3. According to the US NCI plant screening program, a crude extract is generally considered to have *in vitro* cytotoxic activity if the IC_{50} value (concentration that causes reduction in cell viability to 50%) is less than 30 µg/ml (Boik, 2001). Since the IC_{50} concentrations of leaf and stem bark in HT-29 cell line were more than 200 µg/ml, therefore, they are not potent as anticancer therapeutic agent. Moreover, In terms of toxicity to the Chang liver cell, leaf extract was more toxic than stem bark since the IC_{50} of leaf was 199.1 ± 3.90 µg/ml while the value of stem bark was >200 µg/ml.

Cytotoxicity of the root methanolic extract is shown in Figure 9. Root extract appeared to be more active compared to leaf and stem bark on both cell lines. Interestingly, 25 µg/ml of root methanolic extract decreased the HT-29 cell viability to 28.8% while the Chang liver cell viability was 72.4%. The IC_{50} concentration for HT-29 and Chang liver cell lines were 18.3 ± 0.98 and 33.3 ± 0.75 µg/ml respectively (Table 3). Thus, root methanolic extract could be a source of anticancer therapeutic agent against HT-29 cell line. Most anticancer drugs have been discovered through random screening of plant materials. Nowadays, isolation and elucidation of novel compounds have become an important part of cancer research for development of potential anticancer agents (Ma and Wang, 2009). Hence, due to the potential anticancer activity of root methanolic extract, isolation and elucidation of active compounds are recommended.

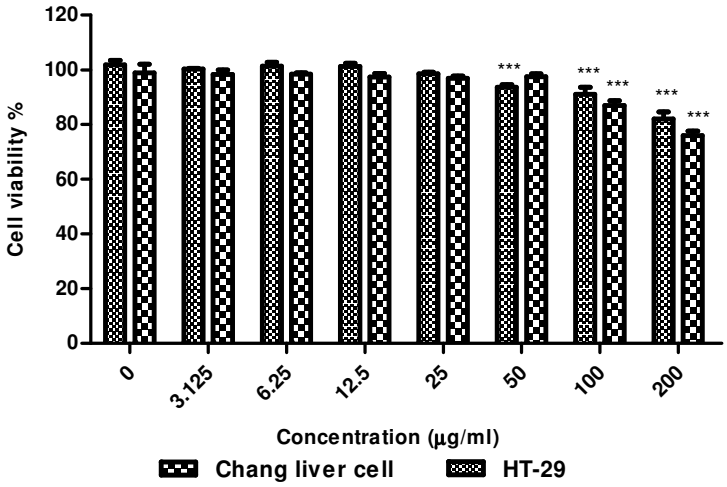

Figure 8. Effect of crude methanolic extract of *J. curcas* stem bark on HT-29 and Chang liver cell viability. All values represent the mean ± S.D from three independent experiments. ***P < 0.001 indicates significant difference compared to the untreated control group.

Table 3. The IC_{50} values of extracts and positive control on HT-29 and Chang liver cell lines.

Sample	IC_{50} value (µg/ml)	
	HT-29	Chang liver cell
Leaf	> 200	199.1 ± 3.90
Stem barks	> 200	> 200
Root	18.3 ± 0.98	33.3 ± 0.75
Latex	70.1 ±2.06	60.1 ± 0.89
Tamoxifen	36.2 ± 2.99	34.4 ± 0.12

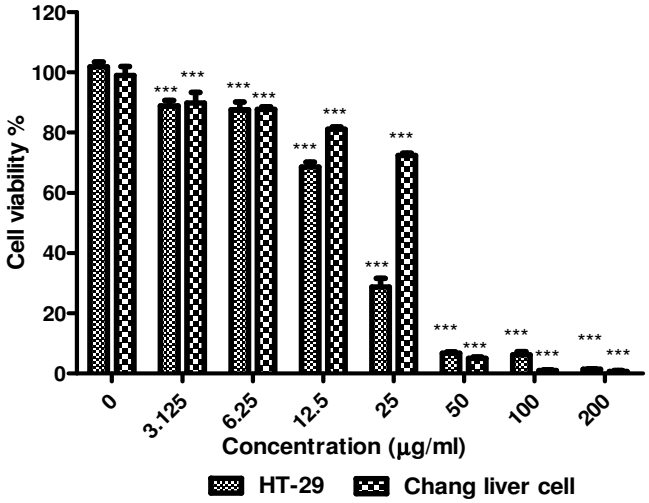

Figure 9. Effect of crude methanolic extract of *J. curcas* root on HT-29 and Chang liver cell viability. All values represent the mean ± S.D. from three independent experiments. ***P < 0.001 indicates significant difference compared to the untreated control group.

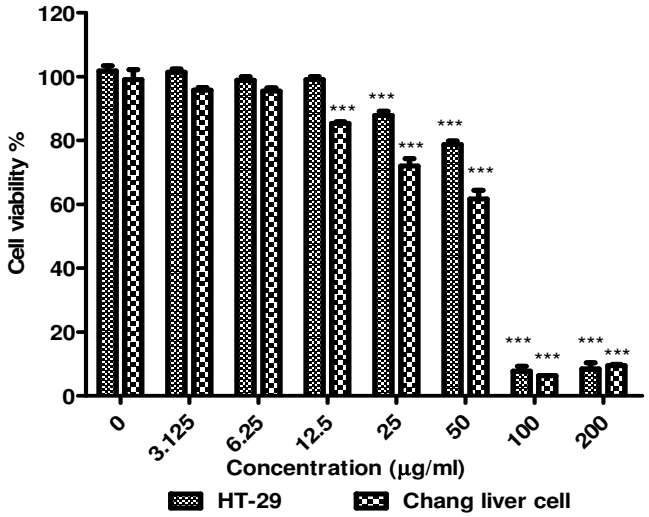

Figure 10. Effect of crude methanolic extract of *J. curcas* latex on HT-29 and Chang liver cell viability. All values represent the mean ± S.D. from three independent experiments. ***P < 0.001 indicate significant difference compared to the untreated control group.

Cytotoxicity of latex extract is shown in Figure 10. It was noted that, the cell viability of both cell lines decreased in a dose-dependent manner. The IC_{50} concentration of latex extract in HT-29 and Chang liver cell were 70.1 ± 2.06 and 60.1 ± 0.89 µg/ml respectively (Table 3). The IC_{50} results suggested that latex was not useful as an anticancer therapeutic agent, due to its high IC_{50} concentration and toxicity to the Chang liver cell.

Tamoxifen was used as a positive control (Figure 5) in

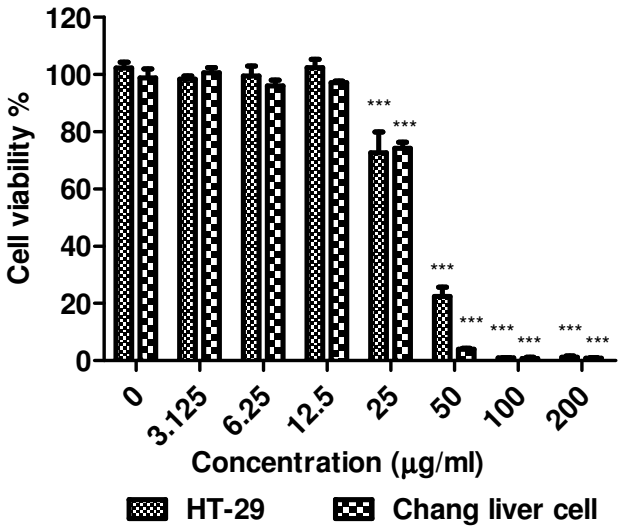

Figure 11. Effect of Tamoxifen on HT-29 and Chang liver cell viability. All values represent the mean ± S.D from three independent experiments. ***P < 0.001 indicates significant difference compared to the untreated control group.

this study. The IC_{50} concentration of Tamoxifen (Table 3) for HT-29 and Chang liver cell were 36.2 ± 2.99 and 34.4 ± 0.12 µg/ml. The activity of Tamoxifen was found to be higher than leaf, stem bark and latex extracts while it was lower than root extract. Tamoxifen has been used specifically to treat breast cancer due to its antagonistic effect on estrogen receptor in breast tissue (Hoskins et al., 2009). However, Kuo et al. (2007) reported the low response of HT-29 cells to Tamoxifen drug. Hence, the low response of Tamoxifen to HT-29 compared to the root extract in the present study, might be due to its mechanism of action which made it specifically active on breast cancer cell compared to HT-29 cell line.

All the extracts showed cytotoxicity effects at various concentrations. Chang liver cell was also susceptible to the extracts, indicating the lack of selectivity in the effect. It should be borne in mind that the present study was based on crude methanolic extracts and detail investigation should be carried out to isolate the bioactive compounds, in particular from the root, that is responsible for the cytotoxicity effect on HT-29 cells. A variety of compounds are present in the plant extracts (phenolic, flavonoid and saponins) and in this condition, the effects can be compounded.

Conclusion

J. curcas Linn. leaf and latex extracts, contained appreciable amounts of phenolic and saponin compounds. These extracts also showed good antioxidant activity towards DPPH and NO radical scavenging activity. Root and latex extracts also actively inhibited the iNOS in macrophages RAW 264.7 cell, induced by LPS and IFN-γ, indicating their potential as anti-inflammatory agent. The cytotoxicity assay indicated the potential of the root as a source of anticancer therapeutic compound.

ACKNOWLEDGMENTS

The authors would like to thank the Ministry of Higher Education for the funds provided under the Fundamental Research Grant Scheme (Project No. 01-11-08-660FR).

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