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*Full Length Research Paper*

# *In vitro* cytotoxic and antioxidant properties of the aqueous, chloroform and methanol extracts of *Dicranopteris linearis* leaves

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**The *in vitro* cytotoxic and antioxidant properties of the aqueous, chloroform and methanol extracts of the *Dicranopteris linearis* leaves were investigated in the present study. The cytotoxic effect was determined against the normal (3T3) and cancer cells' lines (MCF-7, HeLa, HT-29, HL-60, K-562 and MDA-MB-231) using the 3,(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay, while the antioxidant activity was assessed using the DPPH radical and superoxide scavenging assays. Based on the results obtained, the aqueous extract was not effective against any of the types of cancer cells studied; the chloroform extract was effective only against MCF-7 and HeLa; and the methanol extract was effective against all the cancer cells used. Interestingly, all extracts failed to produce cytotoxic effect against the 3T3 cells (normal cell) indicating their safety. All extracts (20, 100 and 500 μg/ml) were found to exert antioxidant activity when tested using the DPPH radical and superoxide scavenging assays; with the methanol, followed by the aqueous and chloroform extracts exhibiting the highest antioxidant activity in both assays. The total phenolic content for the aqueous, methanol and chloroform extracts were 3112.1 ± 6.7, 3417.3 ± 4.7 and 1012.7 ± 5.3 mg/100 g gallic acid, respectively. In conclusion, the leaves of *D. linearis* possess potential cytotoxic activity against various types of cancer cell lines depending on the types of extracts used and antioxidant activity, which need to be further explored.**

**Key words:** *Dicranopteris linearis*, *in vitro* anticancer activity, MTT assay, aqueous extract, chloroform extract, methanol extract.

## INTRODUCTION

Various species of ferns have been claimed to possess enormous economic utility, particularly, their medicinal and food importance (Vasuda 1999). *Dicranopteris linearis* (L.) is a type of ferns that belongs to the family Gleicheniaceae. Known locally to the Malays as '*Resam*', the plant is traditionally used to reduce body temperature and to control fever (Derus 1998; Chin 1992), while in other part of the world, it is used to treat asthma and for woman's sterility (Vasuda 1999), to treat external wound, ulcers and broils and to get rid of intestinal worms (Chin, 1992).

Previously, various types of flavonoids have been successfully isolated and identified from *D. linearis* (Raja et al., 1995) without any attempt to establish its pharmacological activities. Recent study has demonstrated that the chloroform and aqueous extracts of *D. linearis* possessed antinociceptive, anti-inflammatory, antipyretic (Zakaria et al. 2006; 2007a) and antioxidant effects

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(Zakaria, 2007b). The objective of the present study is to determine the *in vitro* anticancer and antioxidant activities of the aqueous, chloroform and methanol extracts of *D. linearis* leaves using various standard experimental assays.

## MATERIALS AND METHODS

### Plant material

The leaves of *D. linearis* were collected from its natural habitat in Shah Alam, Selangor, Malaysia in August-September 2006. The plant has been identified earlier by Mr. Shamsul Khamis, a botanist at the Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia and a voucher specimen (SK 855/05) has been previously deposited at the Herbarium of IBS, UPM.

### Preparation of aqueous, chloroform and methanol extracts of *D. linearis*

The leaves of *D. linearis* were air-dried for 1 - 2 weeks at room temperature (27 ± 2°C). The dried leaves were then ground into small particles, weighed (40 g) and then serially soaked (72 h; room temperature) in distilled water ($dH_2O$), chloroform and methanol in the ratio of 1:20 (w/v). Each of the mixture solutions were collected and filtered using Whatman No. 1 filter paper to obtained the respective supernatants. The aqueous extracts were kept in freezer (-80°C; 48 h) and then subjected to the freeze-drying process. On the other hand, the chloroform and methanol extracts were evaporated (40°C) under reduced pressure to dryness.

The freeze-drying process led to a yield of 2.0 g (5.0%) crude dried aqueous extracts, while the evaporation of the chloroform and methanol extracts of *D. linearis* resulted in a yield of 2.8 g (7.1%) and 2.7 g (6.8%), respectively. All of the crude dried extracts obtained were kept at 4°C and, prior to use, the aqueous extracts (AEDL) were dissolved in $dH_2O$, while the chloroform (CEDL) and methanol (MEDL) extracts were dissolved in dimethyl sulfoxide (DMSO) to make up the required concentrations (100 µg/ml).

### MTT assay (Boehringer Mannheim)

All cell line cultures (MCF-7, HeLa and HT-29) of the American Type Culture Collection (ATCC) were purchased from the Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. They were cultured in Roswell Park Memorial Institute 1640 supplemented with 10% of fetal bovine serum (FBS), 100 IU/ml of penicillin and 100 µg/ml of streptomycin using 25-$cm^2$ flasks, in 5% $CO_2$ incubator at 37°C. The viability of cells was determined with trypan blue reagent. Exponentially growing cells were harvested, counted with haemocytometer and diluted with a particular medium. Cell culture with the concentration of 1 x $10^5$ cells/ml was prepared and was plated (100 µl/well) onto 96-well plates (NUNCTM, Denmark). The diluted ranges of extracts were added to each well and the final concentrations of the test extracts were 12.5, 25, 50, and 100 µg/ml. The proliferative activity was determined using the MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide). The incubation period used was 72 h. After solubilization of the purple formazan crystals were completed, the spectrophotometrical absorbance of the plants extract was measured using an ELISA reader at a wavelength of 550 nm. The cytotoxicity was recorded as the drug concentration causing 50% growth inhibition of the tumour cells ($IC_{50}$ value) using the formula given below:

$$\%\text{ Cell viability} = \frac{\text{OD sample (mean)}}{\text{OD control (mean)}} \times 100\%$$

After the determination of the percentage of cytotoxicity, graphs were plotted with the percentage of cytotoxicities against their respective concentrations.

### Antioxidant assays

### DPPH radical scavenging activity

Assay for DPPH free radical scavenging potential is based on the scavenging activity of stable DPPH free radicals (Carr et al., 1999). Reaction mixtures containing test samples dissolved in ethanol and reacted with 200 µM DPPH (Sigma) in methanolic solution in a 96-well microtiter plate were incubated at 37°C for 30 min. After the reaction, absorbance was then measured at 520 nm, and percent inhibition was calculated.

### Superoxide anion radical scavenging activity

The superoxide anion radical scavenging activity was performed using the method of Okamura et al. (1993) with some modification. This assay is based on the removal rate of xanthine/xanthine oxidase-generated superoxide by measuring the reduction of nitro blue tetrazolium (NBT). The sample solution (0.1 mg/ml) in 5% DMSO was added to 1 ml of a mixture of 0.1 mM xanthine and 0.2 mM NBT (Sigma) in 50 mM potassium phosphate buffer (pH 7.5) containing 0.05 mM EDTA. Xanthine oxidase (0.1 ml) (Sigma, USA; 0.8 unit/mL) diluted in 50 mM phosphate buffer (pH 7.5) was added, and the resulting mixture was incubated at 37°C for 20 min. Addition of 2 ml of 2.5 N HCl to the mixtures terminated the reaction, followed by increase of coloration of NBT, which was measured at 540 mM. The percent of removal rate by sample was calculated relative to the control.

### Phytochemical screening of the AEDL, CEDL and MEDL

Phytochemical screening of the AEDL, CEDL and MEDL was carried out according to the standard screening tests and conventional protocols (Ikhiri et al. 1992).

### Determination of the total phenolic contents of the AEDL, CEDL and MEDL

The total phenolic content of the AEDL, CEDL and MEDL was determined using the Folin–Ciocalteu method as described by Ragazzi and Veronese (1973). Briefly, 1.0 ml of each extract was added to 10.0 ml distilled water and mixed with 2.0 ml of Folin–Ciocalteu phenol reagent (Merck-Schuchardt, Hohenbrun, Germany). The mixture was allowed to stand at room temperature for 5 min and subsequently, 2.0 ml sodium carbonate was added, which resulted in the formation of blue complex. The complex was then measured at 680 nm with catechin used as a standard for the calibration curve. The phenolic compound content was calibrated using the linear equation base on the calibration curve. The content of phenolic compound was expressed as µg catechin equivalent/g dry weight. The dry weight indicated was that of the leaves of *D. linearis*.

### Statistical analysis

Statistical comparisons were carried out using student's t-test.

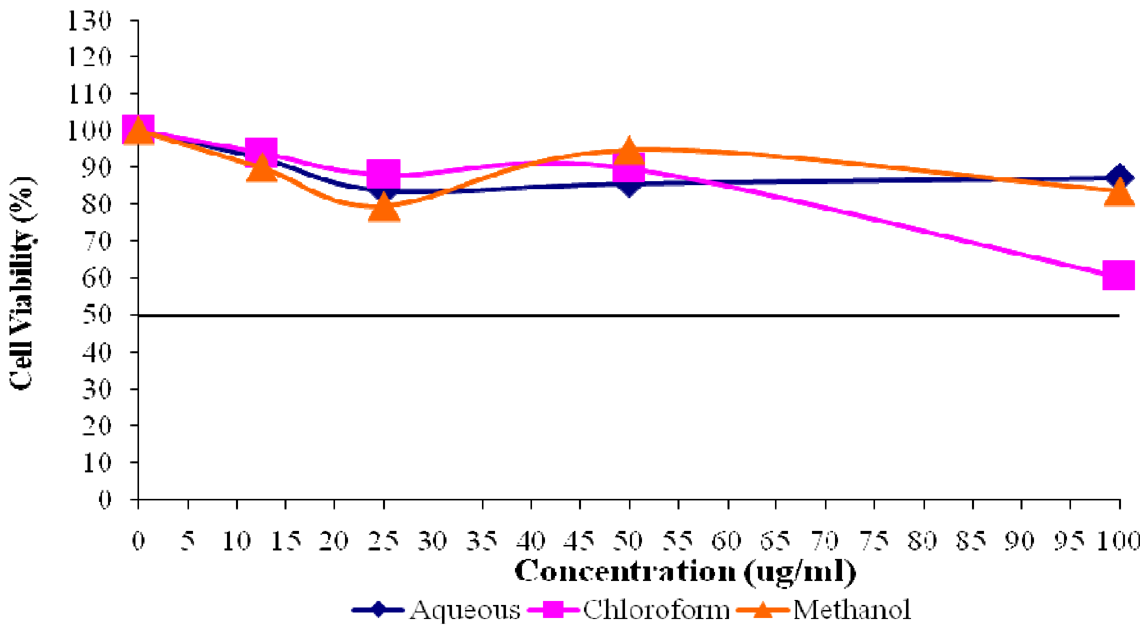


**Figure 1.** *In vitro* antiproliferative activity of AEDL, CEDL and MEDL against 3T3 cancer cell lines.

Probability level of $P < 0.05$ was chosen as the criterion of statistical significance. Values reported were mean ± SD.

## RESULTS

DMSO alone was found to be ineffective in exhibiting antiproliferative activity as indicated by its failure to produce $IC_{50}$ against all of the cell lines used in this study (data not shown).

The antiproliferative activity of the AEDL, CEDL and MEDL against the normal cell line (3T3) is illustrated in Figure 1. Interestingly, all extracts did not produce antiproliferative or cytotoxic effect on the cells. Figure 2 shows the antiproliferative profiles of the AEDL, CEDL and MEDL against the MCF-7 cancer cell line. Only the CEDL and MEDL demonstrated antiproliferative activity against the MCF-7 cells with the $IC_{50}$ values recorded at 92 and 99 µg/ml, respectively. The effect of AEDL, CEDL and MEDL on the proliferative potential of HeLa cancer cell lines is illustrated in Figure 3. Only the CEDL and MEDL were found to inhibit the proliferation of HeLa cells with $IC_{50}$ values of approximately 23 and 64 µg/ml, respectively. Figure 4 shows the antiproliferative profile of the AEDL, CEDL and MEDL against the HL-60 cancer cell line. Only the CEDL and MEDL exerted antiproliferative activity against the HL-60 with $IC_{50}$ values recorded at approximately 27 and 9 µg/ml, respectively.

The antiproliferative profile of the AEDL, CEDL and MEDL against the K-562 cancer cell line is shown in Figure 5. Only the CEDL and MEDL inhibit the proliferation of K-562 cells with $IC_{50}$ values recorded at approximately 88 and 67 µg/ml, respectively. The ability of the AEDL, CEDL and MEDL to inhibit the proliferation of HT-29 cancer cell lines is demonstrated in Figure 6. All extracts of *D. linearis* failed to exert antiproliferative activity against the HT-29 cells.

Interestingly, only MEDL inhibits the proliferation of MDA-MB-231 cells with an $IC_{50}$ value of approximately 40 µg/ml (Figure 7). Comparison in term of $IC_{50}$ was also performed between the extracts of *D. linearis* leaves and tamoxifen, a standard antitumour drug (Table 1). Overall, tamoxifen was a more potent antiproliferative agent compared to the three extracts of both plants as indicated by its lower $IC_{50}$ values.

Table 2 shows the antioxidant activities of AEDL, CEDL and MEDL assessed using the DPPH radical scavenging and superoxide scavenging assays. The 20, 100 and 500 µg/ml MEDL produced the highest antioxidant activity (between 37.8 – 99.7%) in the DPPH radical scavenging test compared to the AEDL (29.3–94.3%) and CEDL (13.3 – 66.1%). MEDL also exhibited the highest activity in the superoxide scavenging test with an activity ranging between 84.2 – 99.7% as compared to the AEDL (62.1 – 97.4%) and CEDL (33.2 – 71.7%).

In addition, the total phenolic content evaluation of the three extracts indicates that MEDL and AEDL contain the highest phenolic content, which is approximately 3417.3 ± 4.7 and 2332.1 ± 5.3 mg/100 g gallic acid, respectively. The CEDL total phenolic content was approximately 1012.7 ± 5.3 mg/100 g gallic acid (Table 3).

The phytochemical screening of the three extracts revealed that AEDL contained saponins, flavonoids, tannins; CEDL contained flavonoids, condensed tannins, triterpenes and steroids and; MEDL contained saponins, flavonoids, condensed tannins and steroids (Table 4).

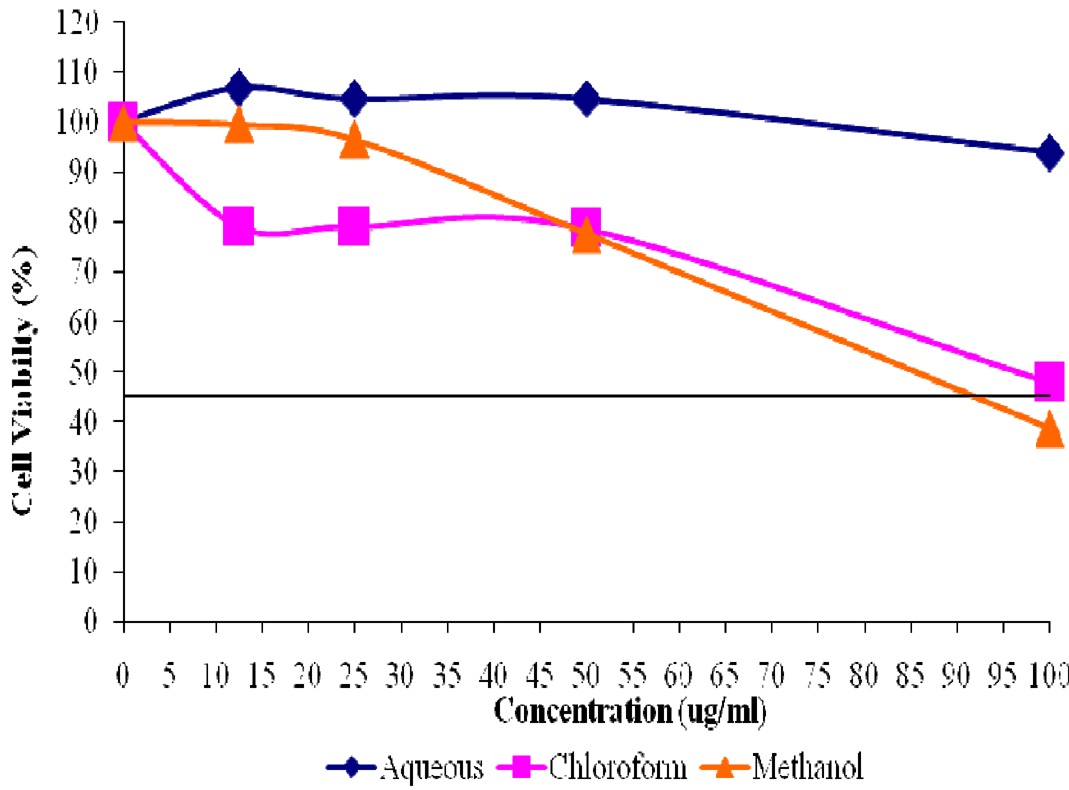


**Figure 2**. *In vitro* antiproliferative activity of AEDL, CEDL and MEDL against MCF-7 cancer cell lines.

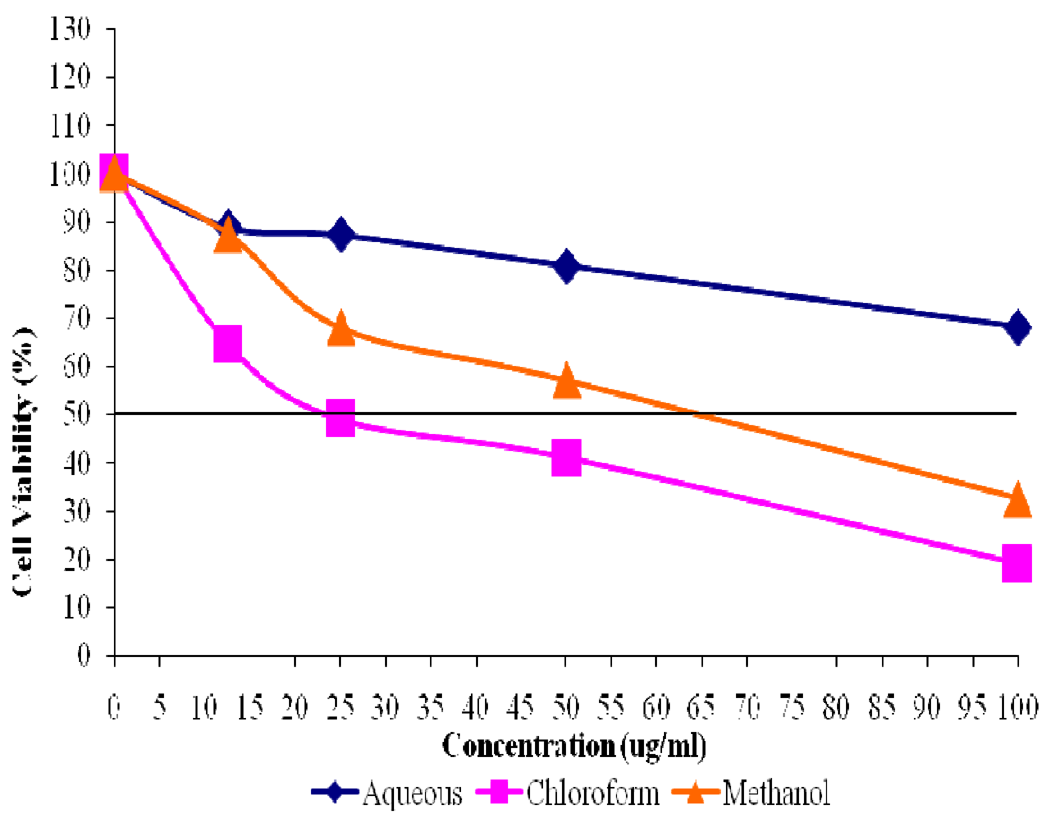


**Figure 3.** *In vitro* antiproliferative activity of AEDL, CEDL and MEDL against HeLa cancer cell lines.

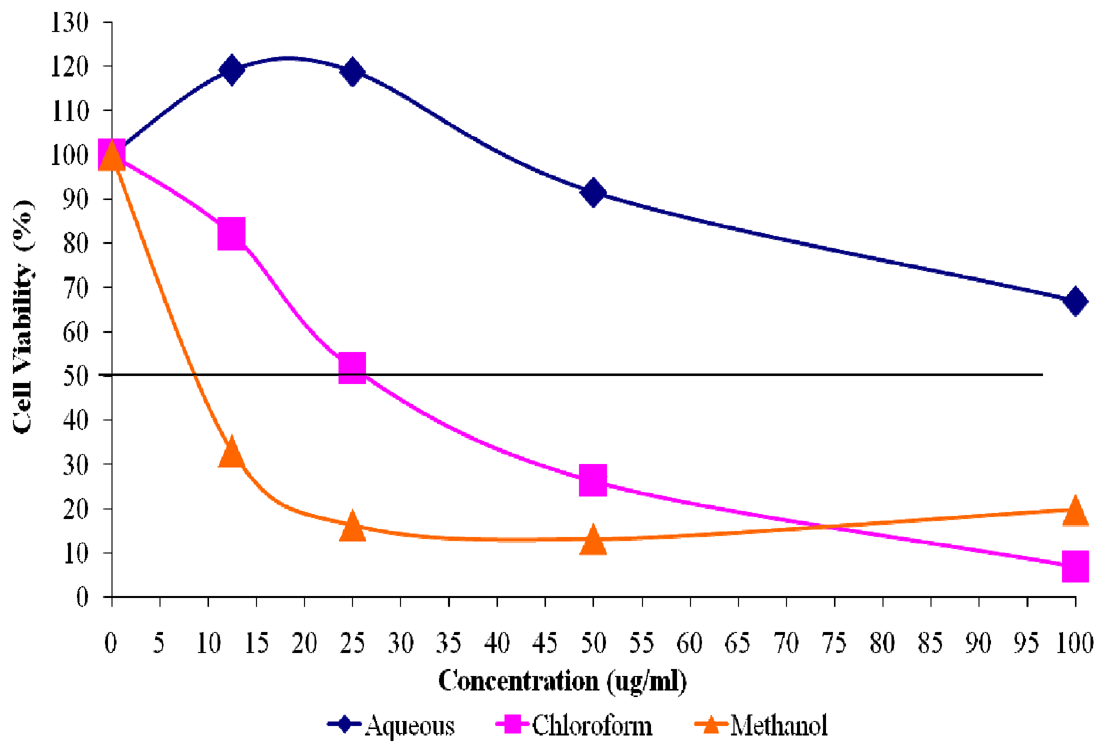


**Figure 4.** *In vitro* antiproliferative activity of AEDL, CEDL and MEDL against HL60 cancer cell lines.

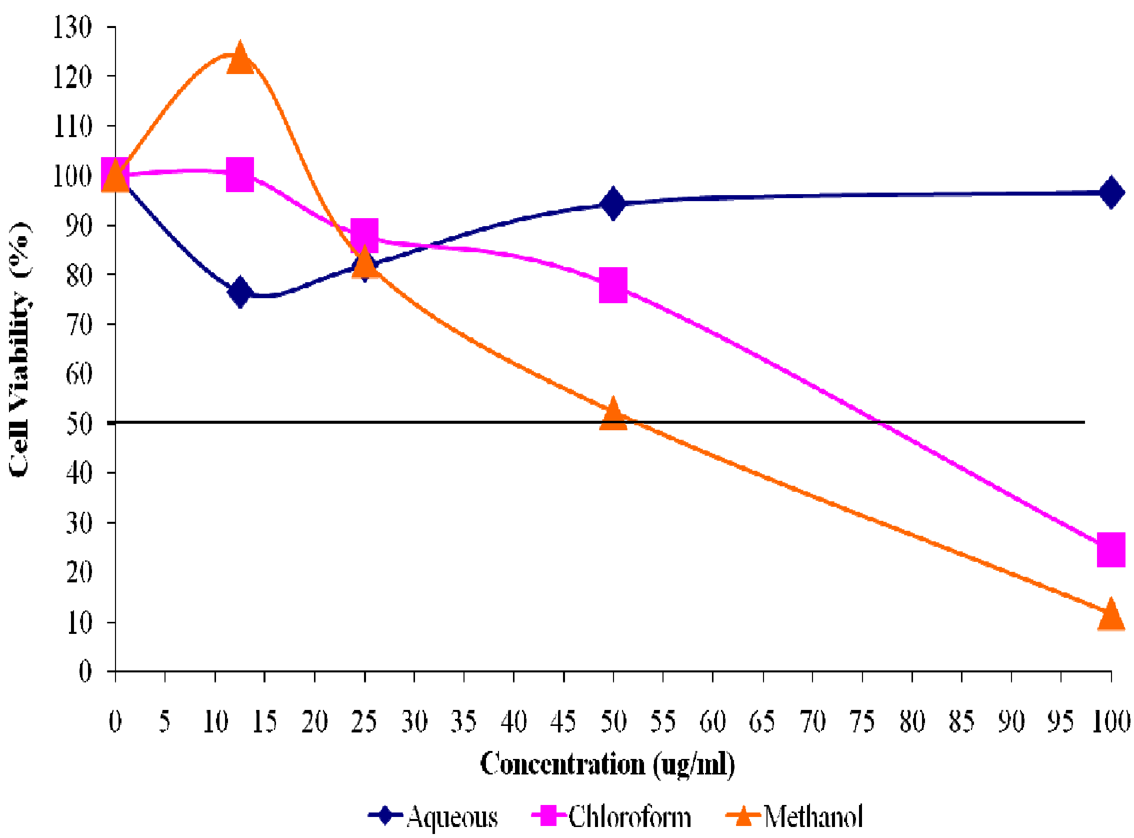


**Figure 5.** *In vitro* antiproliferative activity of AEDL, CEDL and MEDL against K562 cancer cell lines.

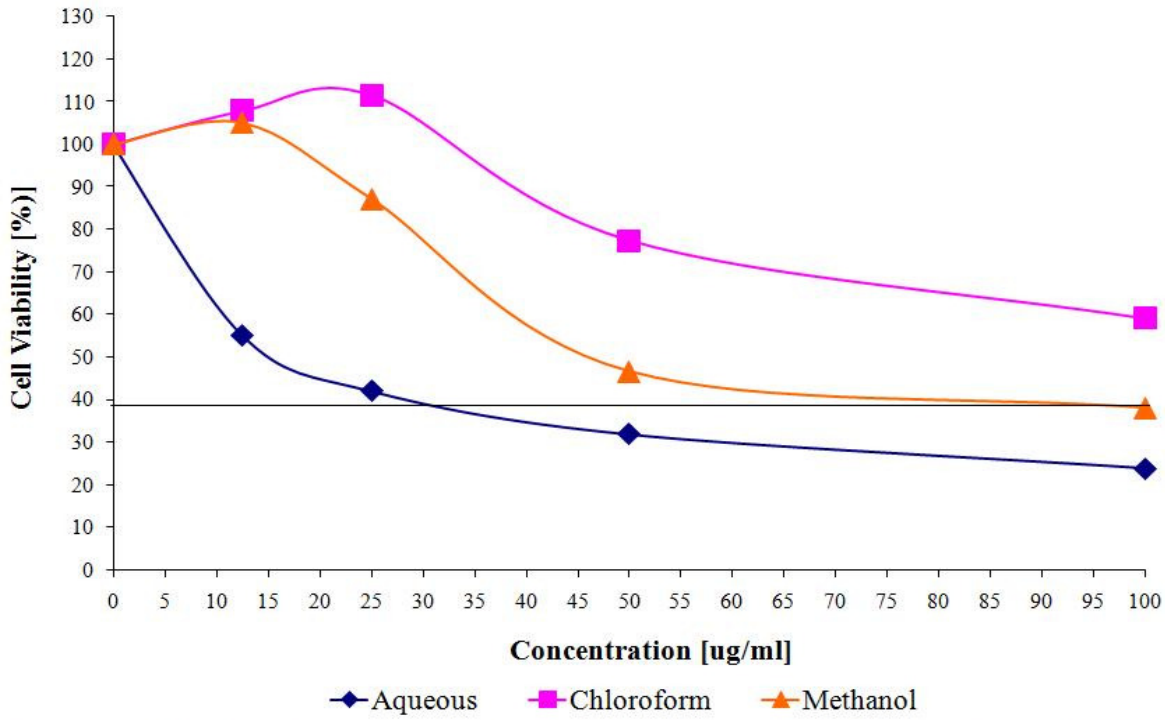


**Figure 6.** *In vitro* antiproliferative activity of AEDL, CEDL and MEDL against HT-29 cancer cell lines.

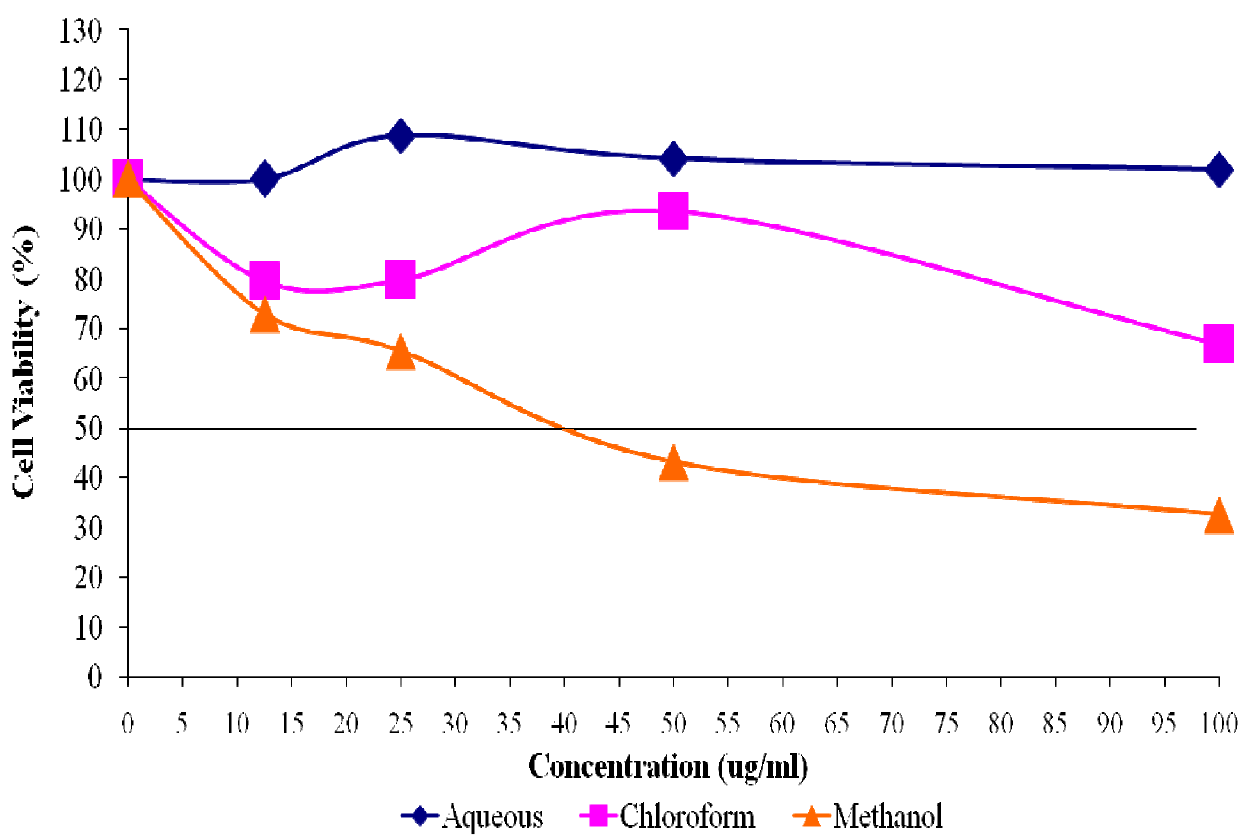


**Figure 7.** *In vitro* antiproliferative activity of AEDL, CEDL and MEDL against MD-MBA-231 cancer cell lines.

**Table 1.** Comparison of the $IC_{50}$ values between tamoxifen and the *D. linearis* extracts.

| Compound/ Extract | $IC_{50}$ values µmg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **3T3** | **MCF-7** | **HeLa** | **HL-60** | **K-562** | **HT-29** | **MDA-MB-231** |
| Tamoxifen | ND | 11[a] | 9[a] | 7[a] | ND | 8[a] | ND |
| AEDL | ND | ND | ND | ND | ND | ND | ND |
| CEDL | ND | 92 | 23 | 27 | 88 | ND | ND |
| MEDL | ND | 99 | 64 | 9 | 67 | ND | 40 |

ND: Not detected because the concentration of extracts/drugs required to exhibit $IC_{50}$ value were above 100 µg/ml (the highest concentration used); [a] Tamoxifen concentration was presented in µM/ml.

**Table 2.** The antioxidant activity of AEDL, CEDL and MEDL assessed by DPPH radical scavenging and superoxide scavenging assays.

| Extract | Concentration (µg/ml) | DPPH radical scavenging (%) | Superoxide scavenging (%) |
|---|---|---|---|
| AEDL | 20 | 29.3 ± 0.8 | 62.1 ± 1.3 |
| | 100 | 61.4 ± 2.1 | 83.0 ± 0.6 |
| | 500 | 94.3 ± 1.2 | 97.4 ± 0.2 |
| CEDL | 20 | 13.3 ± 0.2 | 23.2 ± 0.4 |
| | 100 | 22.6 ± 0.7 | 41.4 ± 0.1 |
| | 500 | 46.1 ± 1.3 | 58.7 ± 0.6 |
| MEDL | 20 | 37.8 ± 1.7 | 84.2 ± 1.3 |
| | 100 | 85.2 ± 0.6 | 96.7 ± 0.9 |
| | 500 | 99.7 ± 0.4 | 99.7± 0.3 |

**Table 3.** The total phenolic content of AEDL, CEDL and MEDL.

| Extract | Concentration (mg/ml) | Total phenolic content (mg/100g Gallic acid) |
|---|---|---|
| AEDL | 6.25 | 3112.1 ± 6.7 |
| CEDL | 6.25 | 1012.7 ± 5.3 |
| MEDL | 6.25 | 3417.3 ± 4.7 |

Total phenolic content (TPC) – Expressed as milligram equivalent of gallic acid per 100 g of dry weight (mg gallic acid/100 g); TPC value > 1000 mg gallic acid/100g is considered as high TPC.

**Table 4.** The phytochemical constituents of the leaves of *M. malabathricum* aqueous, chloroform and methanol extracts.

| Constituent | Extracts of *M. malabathricum* leaves | | |
|---|---|---|---|
| | **Aqueous** | **Chloroform** | **Methanol** |
| Flavonoids | + | + | + |
| Triterpenes | - | - | - |
| Tannins | + | + | + |
| Alkaloids | - | - | - |
| Saponins | + | - | + |
| Steroids | - | + | + |

+: Indicate the presence of respective compound.

**Table 5.** The phytochemical constituents of *D. linearis* leaves.

| Constituent | *D. linearis* |
|---|---|
| Flavonoids | + |
| Triterpenes | + |
| Tannins | + |
| Alkaloids | - |
| Saponins | +++ |
| Steroids | +++ |

For saponins, +: 1 - 2 cm froth; ++: 2 - 3 cm froth; +++: >3 cm froth.
For flavonoids, tannins, triterpenes and steroids, +: weak colour; ++: mild colour; +++: strong colour.
For akalioids, +: negligible amount of precipitate; ++: weak precipitate; +++: strong precipitate.

## DISCUSSION

The present study demonstrated the anticancer profiles of three extract of *D. linearis* whereby AEDL was not effective against any of the types of cancer cells studied; CEDL was effective only against MCF-7 and HeLa; and MEDL was effective against all cancer cells used. Interestingly, all extracts failed to produce cytotoxic effect against the 3T3 cells (normal cell) which indicated their non-toxic property. The MEDL also exhibited the highest antioxidant activity and total phenolic content when assessed using the DPPH radical and superoxide scavenging assays followed by the CEDL and AEDL.

The AEDL and CEDL, particularly, have been demonstrated to exhibit antinociceptive, anti-inflammatory, antipyretic (Zakaria et al., 2006, 2007a) and antioxidant activities (Zakaria, 2007b). The fact that *D. linearis* showed promising antinociceptive, anti-inflammatory and antioxidant activities has triggered the present study. It is well known that there are links between the inflammatory and nociceptive, oxidative and cancer processes and the ability to inhibit any of the processes will definitely lead to the inhibition of the others. For example, nitric oxide (NO) is produced/released under the action of inflammatory stimuli (reactive oxygen species (ROS)) (Olszanecki et al., 2002). Inhibition of ROS leads to the reduction of NO production, which has been demonstrated to lead to the anti-inflammatory, antinociceptive, anticancer and antioxidant activities (Middleton et al., 2000; Robak and Gryglewski, 1988). The free radical scavenging property may be one of the mechanisms by which this plant is effective in the different assays describe earlier and might explain the observed anticancer activity.

Phytochemical screening of *D. linearis* leaves demonstrated the presence of flavonoids, saponins, triterpenes, tannins and steroids, but no alkaloids (Table 5) (Zakaria et al., 2006). Flavonoids, tannins, saponins and triterpenes have all been reported to possess antitumor activity (Ye et al., 2007; Ferguson et al., 2006; Lemeshko et al., 2006; Roy et al., 2006). Flavonoids' anticancer activity has been associated with various mechanisms such as the modulation of cell cycle arrest at the G1/S phase, induction of cyclin-dependent kinase inhibitors, down-regulation of anti-apoptotic gene products, inhibition of cell-survival kinase and inhibition of inflammatory transcription factors (Agarwal et al., 2006) and induction of $Ca^{2+}$-dependent apoptotic mechanism (Sergeev et al., 2006). Saponins have been reported to induce apoptosis by causing permeabilization of the mitochondrial membranes (Lemeshko et al., 2006) or necrotic cell death depending on the types of cancer cells (Russo et al., 2005). Triterpenes were also found to cause cell cycle disruption by decreasing the number of cells in G0/G1 phase, with initial increases in S and G2/M (Roy et al., 2006) or by inhibiting nuclear factor-kappa B (NF-κB) (Lee et al., 2006). The fact that *D. linearis* also exhibited antioxidant activities (Zakaria, 2007b) could also be used to support the present finding that antioxidant and anti-tumor mechanisms are interrelated. Increasing evidences have suggested that many age related human diseases, including cancer, are the result of cellular damage caused by free radicals (Perry et al., 2000; Carr and Frei, 2000). Antioxidants have been shown to play an important role in preventing such diseases. For example, several cancer chemopreventive agents exhibit antioxidant activity through their ability to scavenge oxygen radicals (Ito et al., 1999; Wei and Frenkel, 1993). The present study has also demonstrated the correlation between antioxidant activity observed, total phenolic content and antiproliferative activity recorded for each of the extract, which is in line with report made by Yang et al. (2001). Extracts with high total phenolic content (MEDL) exhibited high antioxidant capacity suggesting that the antioxidant and free radical scavenging properties of MEDL contribute directly or indirectly to their observed antiproliferative activity (Li et al., 2008).

The selectivity of those extracts towards cancer cells, but not normal cells could be due to several factors such as the variety in the coordination of cell cycle's cellular events (Agami and Bernards, 2000; Harper et al., 1993; Kato et al., 1994; Hashemolhosseini et al., 1998; Paulovich et al., 1997; Ferguson et al., 2004) and the ability of the bioactive compounds with potential antiproliferative activity to detect and restore those altered regulatory factors within the cell cycle coordination, which is believed to lead to an effective antitumour activity, and the difference in the membrane composition between cancer and normal cells (Zwaal and Schroit, 1997; Zwaal et al., 2005; Papo et al., 2006).

In terms of the antiproliferative mechanisms involved, several mechanisms could be suggested based on the bioactive compounds presence in each of the extracts. Flavonoids, in particular, have been associated with possible role in the prevention of several chronic diseases involving oxidative stress (Lee et al., 2003) as well as their anticancer (Middleton et al., 2000), antioxidant (Robak and Gryglewski, 1988) and anti-inflammatory activities, *in vitro* and *in vivo* (Calixto et al., 2003; 2004).

Flavonoids have also been reported to modulate the expression of pro-inflammatory gene, such as nitric oxide synthase (NOS) and cyclooxygenase-2 (COX-2) (Dawson and Snyder, 1994; Kim et al., 2004). In terms of the antiproliferative mechanisms used, Agarwal et al. (2006) and Sergeev et al. (2006) have demonstrated that flavonoids were capable of inducing the cyclin-dependent kinase inhibitors or the $Ca^{2+}$-dependent apoptotic mechanism, modulating the cell cycle arrest at the G1/S phase, inhibiting the cell-survival kinase and the inflammatory transcription factors, or down-regulating the anti-apoptotic gene products. Saponins and triterpenes have also been shown to induce apoptosis response on cancer cells, by causing permeabilization of the mitochondrial membranes (Lemeshko et al., 2006), to cause necrotic cell death (Russo et al., 2005) or cell cycle disruption, by decreasing the number of cells in G0/G1 phase, with initial increases in S and G2/M (Roy et al., 2006) or by inhibiting nuclear factor-kappa B (NF-κB) (Lee et al., 2006). Other than that, triterpenes were also reported to exert antitumour activity via the inhibition of tumour-induced angiogenesis (Kimura et al., 2002), while Lin et al. (2003) reported that triterpenes-induced antitu-mour activity involved the suppression of the protein kinase C, activating the p38 mitogen-activated protein (MAP) kinase and c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK), as well as prolonging the G2 cell-cycle phase in Huh-7 cells.

Despite the claims on links between the anti-inflammatory, anti-oxidant and anticancer mechanisms discussed earlier (Olszanecki et al., 2002), the failure of AEDL to show any cytotoxic effect against all the cancer cells tested seems to contradict our earlier findings on its anti-inflammatory and antioxidant activity. Thus, it is suggested that AEDL uses mechanism of anti-inflammatory activity that is different from the mechanism of anticancer. For example, the AEDL might only inhibit cyclo-oxygenase (COX) action or prostaglandin synthesis instead of inhibiting the inflammatory stimuli (NO and NOS) (Olszanecki et al., 2002) or inflammatory transcription factors (Agarwal et al., 2006). However, further in-depth studies are required before final conclusion on the mechanisms involved could be drawn to explain the observed activity. In conclusion, the extracts of *D. linearis* leaves possess anticancer activity against various types of cancer cells and antioxidant activity that are correlated to their total phenolic content.

## REFERENCES


Agami R, Bernards R (2000). Distinct initiation and maintenance mechanisms cooperate to induce $G_1$ cell cycle arrest in response to DNA damage. Cell, 102: 55-66.

Agarwal R, Agarwal C, Ichikawa H, Singh RP, Agarwal BB (2006). Anticancer potential of silymarin: from bench to bed side. Anticancer Res. 26: 4457-4498.

Calixto JB, Campos MM, Otuki MF, Santos ARS (2004). Anti-inflammatory compounds of plant origin. Part II. Modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. Planta Med. 70: 93-103.

Calixto JB, Otuki MF, Santos ARS (2003). Anti-inflammatory compounds of plant origin. Part I. Action on arachidonic acid pathway, nitric oxide and nuclear factor κ-B (NFκB). Planta Med. 69: 973-983.

Carr A, Frei B (2000). The role of natural antioxidants in preserving the biological activity of endothelium-derived nitric oxide. Free Rad. Biol. Med. 28: 1806-1814.

Carr Y, Wong M, Rosen RT, Ho CT (1999). 2,2-Diphenyl-1-picrylhydrazyl radical scavenging active components from *Polygonum multiflorum* Thunb. J. Agric. Food Chem. 47: 2226-2228.

Chin WY (1992). A Guide to Medicinal Plants. Singapore Science Centre, p. 24.

Dawson TM, Snyder SH (1994). Gases as biological messengers: Nitric oxide and carbon monoxide in the brain. J. Neurosci. 14: 5147-5159.

Derus ARM (1998). Pengenalan dan Penggunaan Herba Ubatan. Kuala Lumpur, Multiple Triple Vision, p. 75.

Ferguson PJ, Kurowska E, Freeman DJ, Chambers AF, Koropatnick DJ (2004). A flavonoid fraction from cranberry extract inhibits proliferation of human tumor cell lines. J. Nutr. 134: 1529-1535.

Ferguson PJ, Kurowska EM, Freeman DJ, Chambers AF, Koropatnick J (2006). *In vivo* inhibition of growth of human tumor lines by flavonoid fractions from cranberry extract. Nutr. Cancer, 56: 86-94.

Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ (1993). The p21 cdk-interacting protein cip1 is a potent inhibitor of $G_1$ cyclin-dependent kinases. Cell, 75: 805-816.

Hashemolhosseini S, Nagamine Y, Morley SJ, Desrivieres S, Mercep L, Ferrari S (1998). Rapamycin inhibition of the $G_1$ to S transition is mediated by effects on cyclin D1 mRNA and protein stability. J. Biol. Chem. 273: 14424-14429.

Ikhiri K, Boureima D, Dan-Kouloudo D (1992). Chemical screening of medicinal plants used in the traditional pharmacopoeia of Niger. Int. J. Pharmacog. 30: 251-262.

Ito H, Miyake M, Nishitani E, Mori K, Hatano T, Okuda T, Takasaki M, Nishino H, Yoshida T (1999). Antitumor promoting activity of polyphenols from *Cowania mexicana* and *Coleogyne ramosissima*. Cancer Lett. 143: 5-13.

Kato JY, Matsuoka M, Polyak K, Massague J, Sherr CJ (1994). Cyclic AMP-induced $G_1$ phase arrest mediated by an inhibitor ($p27^{kip1}$) of cyclin-dependent kinase 4 activation. Cell, 79: 487-496.

Kim HP, Son KH, Chang HW Kang SS (2004). Anti-inflammatory plant flavonoids and cellular action mechanisms. J. Pharmacol. Sci. 96: 229-245.

Kimura Y, Taniguchi M, Baba K (2002). Antitumor and antimetastatic effects on liver of triterpenoid fractions of *Ganoderma lucidum*: Mechanism of action and isolation of an active substance. Anticancer Res. 22: 3309-3318.

Lee JH, Koo TH, Yoon H, Jung HS, Jin HZ, Lee K, Hong YS, Lee JJ (2006). Inhibition of NF-kappa B activation through targeting I kappaB kinase by celastrol, a quinone methide triterpenoid. Biochem. Pharmacol. 72: 1311-1321.

Lee KW, Kim YJ, Lee HJ, Lee CY (2003). Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. J. Agric. Food. Chem. 51: 7292-7295.

Lemeshko VV, Haridas V, Quijano Perez JC, Gutterman JU (2006). Avicins, natural anticancer saponins, permeabilize mitochondrial membranes. Arch. Biochem. Biophys. 454: 114-122.

Li F, Wang F, Yu F, Fang Y, Xin Z, Yang F, Xu J, Zhao L, Hu Q (2008). *In vitro* antioxidant and anticancer activities of ethanolic extract of selenium-enriched green tea. Food Chem. 111: 165-170.

Lin SB, Li CH, Lee SS, Kan LS (2003). Triterpene-enriched extracts from *Ganoderma lucidum* inhibit growth of hepatoma cells via suppressing protein kinase C, activating mitogen-activated protein kinases and G2-phase cell cycle arrest. Life Sci. 72: 2381-2390.

Middleton Jr. E, Kandaswami C, Theoharides TC (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol. Rev. 52: 673-751.

Okamura H, Mimura A, Yakou Y, Niwano M, Takahara Y (1993). Antioxidant activity of tannins and flavonoids in *Eucalptus rostrata*. Phytochemistry, 33: 557-561.

Olszanecki R, Gêbska A, Kozlovski VI, Gryglewski RJ (2002). Flavonoids and nitric oxide synthase. J. Physiol. Pharmacol. 53: 571-

584.
Papo N, Seger D, Makovitzki A., Kalchenko V, Eshhar Z, Degani H, Shai Y (2006). Inhibition of tumor growth and elimination of multiple metastases in human prostate and breast xenografts by systemic inoculation of a host defense-like lytic peptide. Cancer Res. 66: 5371-5378.
Paulovich AG, Toczyski DP, Hartwell LH (1997). When checkpoints fail. Cell, 88: 315-321.
Perry G, Raina AKL, Nonomura A, Wataya T, Sayre LM, Smith MA (2000). How important is oxidative damage? Lessons from Alzheimer's disease. Free Rad. Biol. Med. 28: 831-834.
Ragazzi E, Veronese G (1973). Quantitative analysis of phenolics compounds after thin-layer chromatographic separation. J. Chromatogr. 77: 369-375.
Raja DP, Manickam VS, De Britto AJ, Gopalakrishnan S, Ushioda T, Satoh M, Tanimura A, Fuchino H, Tanaka N (1995). Chemical and chemotaxonomical studies on Dicranopteris species. Chem. Pharm. Bull. 43: 1800-1803.
Robak J, Gryglewski RJ (1988). Flavonoids are scavengers of superoxide anions. Biochem. Pharmacol. 37: 837-841.
Roy MK, Kobori M, Takenaka M, Nakahara K, Shinmoto H, Isobe S, Tsushida T (2006). Antiproliferative effect on human cancer cell lines after treatement with nimbolide extracted from an edible part of the neem tree (*Azadirachta indica*). Phytother. Res. 21: 245-250.
Russo A, Cardile V, Lombardo L, Vanella L, Vanella A, Garbarino JA (2005). Antioxidant activity and antiproliferative action of methanolic extract of *Geum quellyon* Sweet roots in human tumor cell lines. J. Ethnopharmacol. 100: 323-332.
Sergeev IN, Li S, Colby J, Ho CT, Dushenkov S (2006). Polymethoxylated flavones induce Ca(2+)-mediated apoptosis in breast cancer cells. Life Sci. 80: 245-253.
Vasuda SM (1999). Economic importance of pteridophytes. Indian Fern. J. 16: 130-152.
Wei HC, Frenkel K (1993). Relationship of oxidative events and DNA oxidation in SENCAE mice to *in vivo* promoting activity of phorbol ester-type tumor promoter. Carcinogen, 14: 1195-1201.
Yang CS, Landau JM, Huang MT, Newmark HL (2001). Inhibition of carcinogenesis by dietary polyphenolic compounds. Annu. Rev. Nutr. 21: 381-406.
Ye CL, Liu Y, Wei DZ (2007). Antioxidant and anticancer activity of 3'-formyl-4', 6'-dihydroxy-2'-methoxy-5'-methylchalcone and (2S)-8-formyl-5-hydroxy-7-methoxy-6-methylflavanone. J. Pharm. Pharmacol. 59: 553-559.
Zakaria ZA (2007b). Free radical scavenging activity of some plants available in Malaysia. Iran. J. Pharmacol. Ther. 6: 87-91.
Zakaria ZA, Abdul Ghani ZDF, Raden Mohd Nor RNS, Hanan Kumar G, Sulaiman MR, Fatimah CA (2006). Antinociceptive and anti-inflammatory activities of *Dicranopteris linearis* leaves chloroform extract in experimental animals. Yakugaku Zasshi, 126: 1197-1203.
Zakaria ZA, Abdul Ghani ZDF, Raden Mohd Nor RNS, Hanan Kumar G, Sulaiman MR, Mat Jais AM, Somchit MN, Arifah AK, Ripin J (2007a). Antinociceptive, anti-inflammatory and antipyretic properties of *Dicranopteris linearis* leaves aqueous extract in experimental animals. J. Nat. Med. 62: 179-187.
Zwaal RF, Comfurius P, Bevers EM (2005). Surface exposure of phosphatidylserine in pathological cells. Cell Mol. Life Sci. 62: 971-988.
Zwaal RF, Schroit AJ (1997). Pathophysiologic implications of membrane phospholipid asymmetry in blood cells. Blood, 89: 1121-1132.