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Full Length Research Paper

Bauhinia purpurea leaves' extracts exhibited *in vitro* antiproliferative and antioxidant activities

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The antiproliferative and antioxidant activities of various extracts of the leaves of *Bauhinia purpurea* were studied using *in vitro* standard assays. The aqueous and chloroform extracts successfully inhibited the proliferation of all cancer cells while the methanol extract inhibited the proliferation of all cells except the CEMss cells when assessed using the 3,(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay. The aqueous extract was effective against MCF-7 (IC_{50} ≈ 9 µg/ml), MDA-MB 231 (IC_{50} ≈ 17 µg/ml) and Caov-3 (IC_{50} ≈ 16 µg/ml); the chloroform extract was highly effective against the CEMss (IC_{50} ≈ 18 µg/ml) and HeLa (IC_{50} ≈ 21 µg/ml); and the methanol extract was highly effective only against the HL-60 (≈ 12 µg/ml) cell lines. Interestingly, all extracts did not inhibit the proliferation of 3T3 cells suggesting their non-cytotoxic properties. The aqueous and methanol, but not chloroform, extracts of *B. purpurea* (20, 100 and 500 µg/ml) exhibited concentration-dependent antioxidant activity only in the superoxide scavenging assay, but low to moderate activity in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, which could be associated with their total phenolic contents. In conclusion, the *B. purpurea* leaf possesses potential antiproliferative and concentration-dependent antioxidant activities. Purification and determination of active compounds are required for further study.

Key words: *Bauhinia purpurea*, *in vitro*, antiproliferative activity, antioxidant activity, phenolic compounds.

INTRODUCTION

Herbal remedies are often sought by patients with chronic disease especially patients with cancers to provide symptom relief (Saydah and Eberhardt, 2002; Barnes et al., 2004; Mao et al., 2007). Barnes et al. (2004) have also reported that, natural product was one of the therapies most commonly used by adults and children in the U.S. Increase in death associated with cancer (Izevbigie, 2003), the callous side effects of some of the cancer chemotherapies (Humpel and Jones, 2006) and the recurrence of drug-resistant tumors as well as the lack of selectivity of anticancer drugs (Ferguson et al., 2004), have triggered the search for more natural cancer fighting agents, particularly those derived from plants. The need for a continuous search for novel natural products that can act as cancer chemopreventive and/or chemotherapeutic agents, have triggered an increase in the interest on plant-derived secondary metabolites, with potential anticancer activity (Harborne, 2000). A variety of edible plants and compounds isolated from them have exerted anticancer activity.

One of the plants that are currently under investigation in our laboratory is *Bauhinia purpurea* (family

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Abbreviations: ROS, Reactive oxygen species; **MTT,** 3,(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay; **DMSO,** dimethyl sulfoxide; **FBS,** fetal bovine serum; **BHT,** butylated hydroxytoluene; **NBT,** nitro blue tetrazolium; **TPC,** total phenolic content; **NF-κB,** nuclear factor-kappa B; **NOS,** nitric oxide synthase.

Leguminoseae). Known to the Malays as '*pokok tapak kerbau*', *B. purpurea* is native to Southern China and India, and have been used to treat stomach tumors, ulcers, wounds, glandular swellings, diarrhea and fever (Zakaria et al., 2007). Scientifically, we have reported on the antinociceptive and anti-inflammatory activities of the aqueous extract of *B. purpurea* leaves and phytochemistry study has also revealed the presence of flavonoids, triterpenes, tannins and steroids (Zakaria et al., 2007). Different parts of *B. purpurea* contained kaempferol, quercetin and isorhamnetin (Salatino et al., 1999) havepacharin and bauhiniastatins 1-4 (Yadava and Tripathi, 2000) and dihydrodibenzoxepins (1-8), a dihydrobenzofuran, a novel spirochromane-2,1'-hexenedione and a new bibenzyl (Boonphong et al., 2007). Bauhiniastatins 1-4 were later found to exhibit anticancer activity against a minipanel of human cancer cell lines, including P388 lymphocytic leukemia cell line. Furthermore, Boonphong et al. (2007) demonstrated that, some of the isolated compounds exerted antimycobacterial, antimalarial, antifungal, cytotoxic and anti-inflammatory activities. Previous findings by Panda and Kar (1999) have demonstrated the ability of *B. purpurea* bark extract to stimulate thyroid function in female mice *via* increasing serum triiodothyronine (T3) and thyroxine (T4) concentrations as well as increasing the hepatic glucose-6-phosphatase and antiperoxidative activities. Recent findings have revealed the ability of *B. purpurea* bark and leaf extracts to ameliorate metformin-induced hypothyroidsm in Type II diabetic mice (Jatwa and Kar, 2009). In another study, it was shown to exhibit wound healing effect on experimentally induced excision, incision, burn and dead space wound models in Sprague Dawley rats (Ananth et al., 2010). In addition, Lakshmi et al. (2009) have also reported the potential of *B. purpurea* leaf and unripe pod to exert protective effects against gentamicin-induced nephrotoxicity in rats. There are also patents related to *B. purpurea* wherein its lectin derivatives were used as larvicides against insects such as European corn borer (Rao et al., 1999). Wirth and Buchholz (2008) claimed that, the composition comprising an aqueous or hydroalcoholic extract of various types of *Bauhinia*, including *B. purpurea* are useful for care, preservation or improvement of the general state of the hair and skin, for prevention of human hair and skin ageing processes, and for the treatment of diseases associated with skin ageing.

Reactive oxygen species (ROS) play important roles in the mechanisms of inflammation/pain, oxidation and cancers (Middleton et al., 2000). Inhibition of ROS has been claimed to be one of the mechanisms of anti-inflammatory/antinociceptive, anticancer and antioxidant activities (Repetto and Llesuy, 2002). Based on these claims and our recent findings on the plant anti-inflammatory and antinociceptive activities, the present study was designed to study the antiproliferative and antioxidant activities of the various extracts of *B. purpurea* using the 3,(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay and the DPPH radical scavenging and superoxide anion scavenging assays, respectively.

MATERIALS AND METHODS

Plants materials

The leaves of *B. purpurea* were collected in August to September 2007 from their natural habitat in Shah Alam, Selangor, Malaysia and were identified by Mr. Shamsul Khamis, a botanist at the Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia. A voucher specimen (SK 1095/05) has been deposited at the Herbarium of the Laboratory of Natural Products, Institute of Bioscience, UPM, Serdang, Selangor, Malaysia.

Preparation of the aqueous, chloroform and methanol extracts of *B. purpurea* leaves

The matured leaves of *B. purpurea* were air dried for 1 to 2 weeks at room temperature (27 ± 2°C) according to previous studies (Zakaria et al., 2006a; 2006b). The dried leaves were then grinded into small particles, weighed (40 g) and then sequentially soaked at room temperature for 72 h with 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 aqueous, chloroform and methanol supernatants. The aqueous extracts of *B. purpurea* was kept at -80°C for at least 48 h and then subjected to the freeze-drying process leading to a yield of 2.1 g (5.2%) of crude extracts, while the chloroform and methanol extracts of the plants were evaporated at 40°C under reduced pressure to dryness resulting in a yield of 2.7 g (6.6%) and 1.1 g (2.7%), respectively. All the dried crude extracts obtained were kept at 4°C and prior to use, the aqueous extracts were dissolved in dH_2O while the chloroform and methanol extracts were dissolved in 100% dimethyl sulfoxide (DMSO) to prepare the respective stock solutions (10 mg/ml).

MTT assay

All cell lines cultures of the American Type Culture Collection (ATCC), namely 3T3 (normal mouse fibroblast), MCF-7 (estrogen-dependent human breast adenocarcinoma), MDA-MB-231 (human breast carcinoma), Caov-3 (human ovarian adenocarcinoma), HL-60 (acute promyelocytic leukemia), CEMss (T-lymphoblastic leukemia) and HeLa (human cervical adenocarcinoma) were purchased from the American Type Culture Collection (ATCC; Rockville, MD) MCF-7 and HT-29 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 a concentration of 1 x 10^5 cells/ml was prepared and plated (100 µl/well) onto 96 well plates (NUNCTM, Denmark). Prior to addition of cells onto the plates, the stock solution was diluted with media, transferred onto the wells and sequentially added with cells in media to achieve the required starting concentration of 100 µg/ml in 1% DMSO. The concentration was serially diluted in each well to achieve concentration range of 100 - 12.5 µg/ml. The proliferative activity was determined using MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Abdullah Sani et al., 2004). The incubation period was 72 h. The spectrophotometrical absorbance of the mammalian cell extract was measured using an enzyme-linked immunosorbent assay (ELISA)

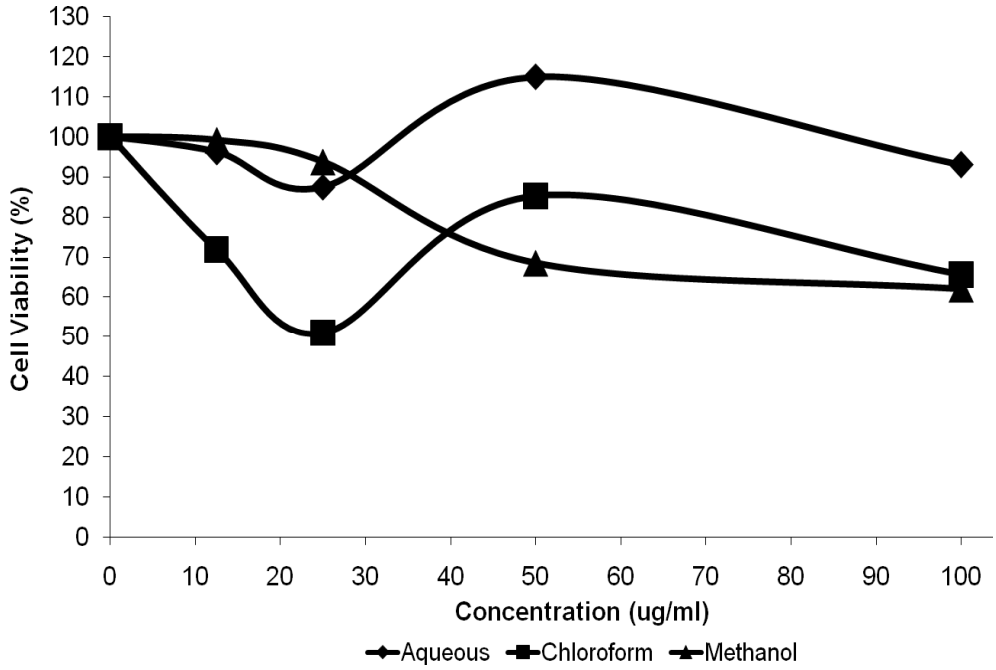

Figure 1. *In vitro* antiproliferative activity of the aqueous, chloroform and methanol extracts of *B. purpurea* leaves against 3T3 normal cell lines.

reader at a wavelength of 550 nm after solubilization of the purple formazan crystals. Cytotoxicity was recorded as the drug concentration causing 50% growth inhibition of the tumour cells (IC_{50} value) using the formula given below:

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

Where, OD = optical density.

After determining the percentage of cytotoxicity, graphs were plotted against its respective concentrations. In all the experiments, the antiproliferative assay was repeated three times with tamoxifen as the standard antitumour drug.

Antioxidant assays

DPPH radical scavenging activity

Assay for DPPH free radical scavenging potential was conducted using previous method with some modifications (Chen et al., 1999). Reaction mixtures containing test samples in methanol and 200 µM DPPH (Sigma) in ethanolic solution were incubated at 37°C for 30 min in a 96 well microtiter plate. After the reaction, absorbance was then measured at 520 nm and percentage of inhibition was calculated. Butylated hydroxytoluene (BHT) was used as positive control.

Superoxide anion radical scavenging activity

The superoxide anion radical scavenging activity was performed using the method of Okamura et al. (1993) with some modifications. This assay is based on the rate of removal 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, USA) 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 in the coloration of NBT, which was measured at 540 mM. The percentage of rate of superoxide removal by sample was calculated relative to the control. Quercetin was used as positive control.

Phytochemical screening of the various extracts of the *B. purpurea* leaves

Phytochemical screening was carried out on the aqueous, chloroform and methanol extracts of *B. purpurea* leaves according to the standard screening tests and conventional protocols as described by Ikhiri et al. (1992).

RESULTS

DMSO alone did not inhibit proliferative activity of all the cell lines used in the present study, which is as indicated by its failure to produce the respective IC_{50} against all of the cell lines. The aqueous, chloroform and methanol extracts of *B. purpurea* leaves did not affect the percentage of cells viability when tested against the normal cell line (3T3) (Figure 1). The percentages of viability of 3T3 cells recorded were above 60%, indicating its non-toxic properties.

Figure 2 shows the antiproliferative profiles of the aqueous, chloroform and methanol extracts of *B. purpurea*

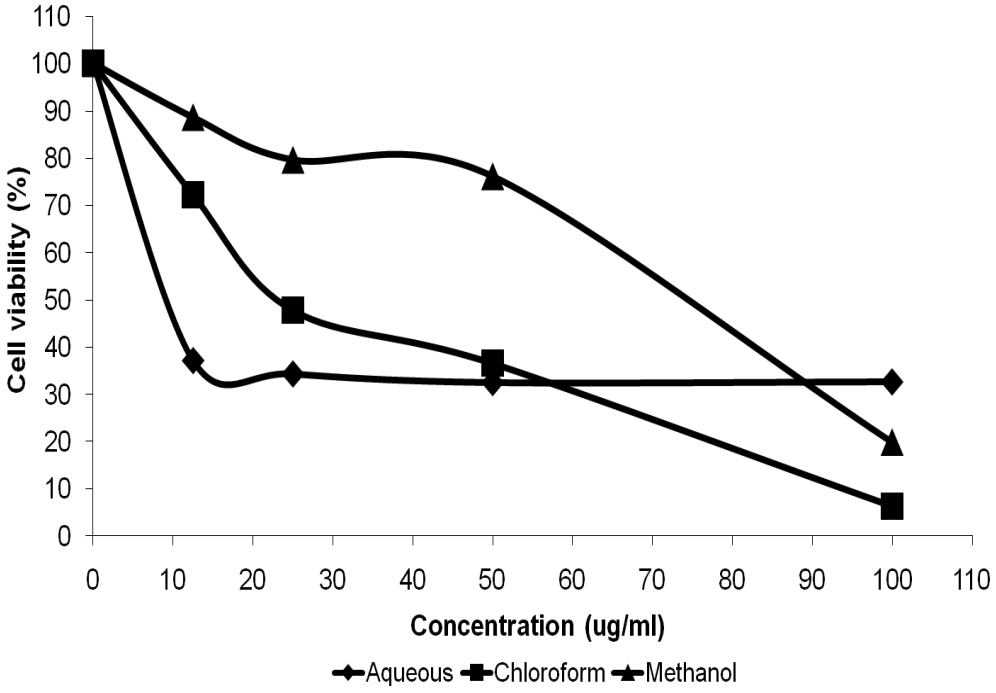

Figure 2. *In vitro* antiproliferative activity of the aqueous, chloroform and methanol extracts of *B. purpurea* leaves against MCF-7 cancer cell lines.

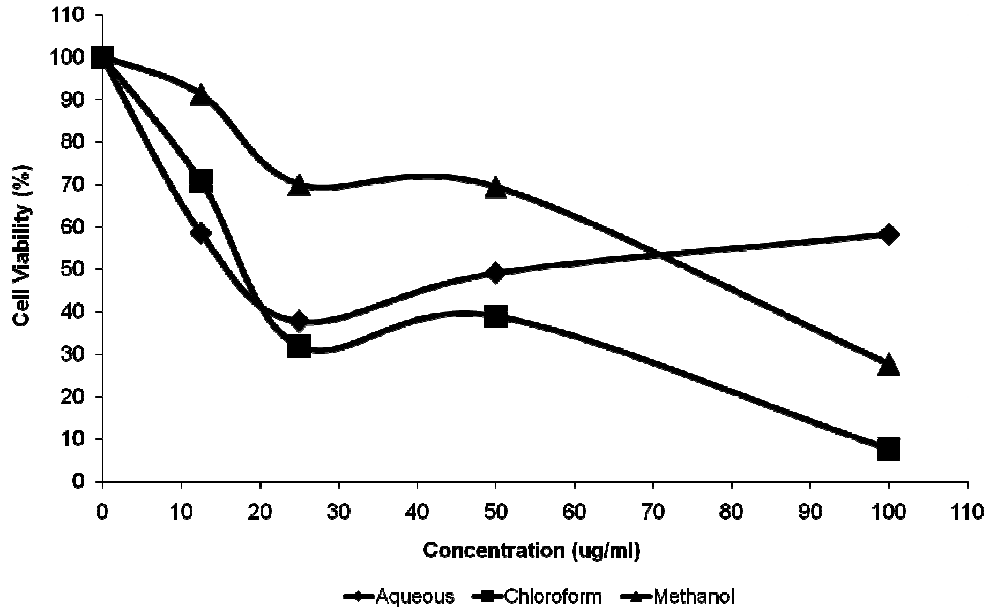

Figure 3. *In vitro* antiproliferative activity of the aqueous, chloroform and methanol extracts of *B. purpurea* leaves against MDA-MB-231 cancer cell lines.

leaves against the MCF-7 cancer cell line. All extracts inhibited the proliferation of MCF-7 with the effective antiproliferative activity seen in the order of aqueous extract followed by the chlororform and methanol extracts. The IC_{50} values recorded for these extracts were 9, 23 and 83 µg/ml, respectively.

All extracts of *B. purpurea* leaves exhibited significant antiproliferative activity against the MDA-MB 231 cancer cell line as shown in Figure 3. The aqueous extract followed by the chloroform and methanol extracts exerted their activities at IC_{50} values of 17, 19 and 75 µg/ml, respectively. The ability of the aqueous, c hloroform and

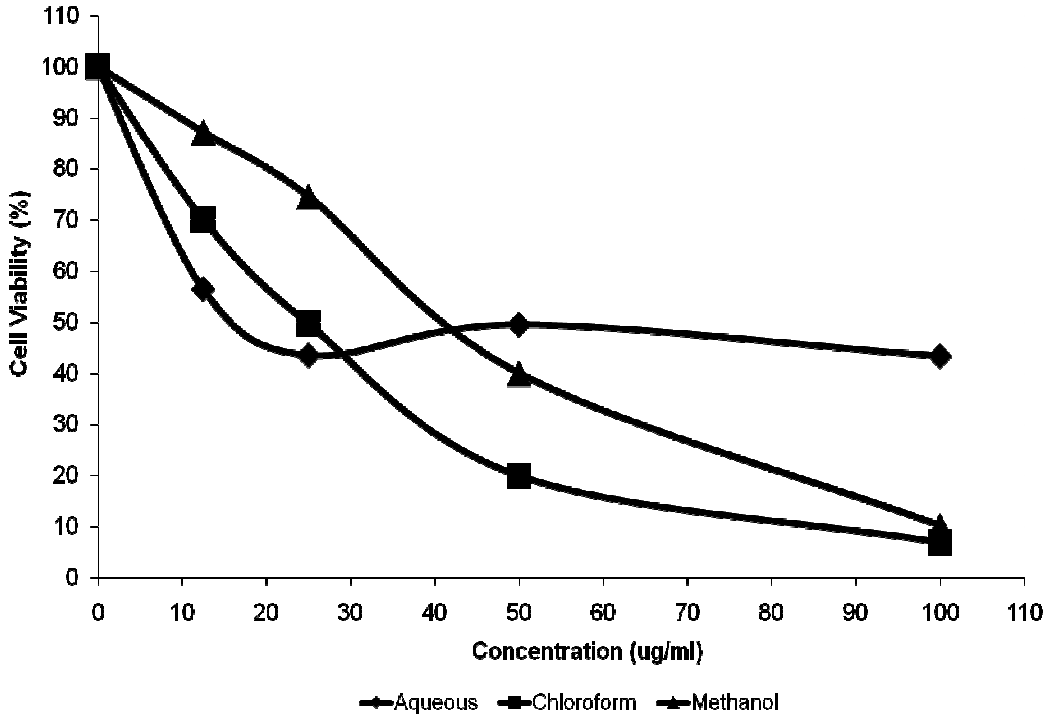

Figure 4. *In vitro* antiproliferative activity of the aqueous, chloroform and methanol extracts of *Melastoma malabathricum* leaves against CaoV3 cancer cell lines.

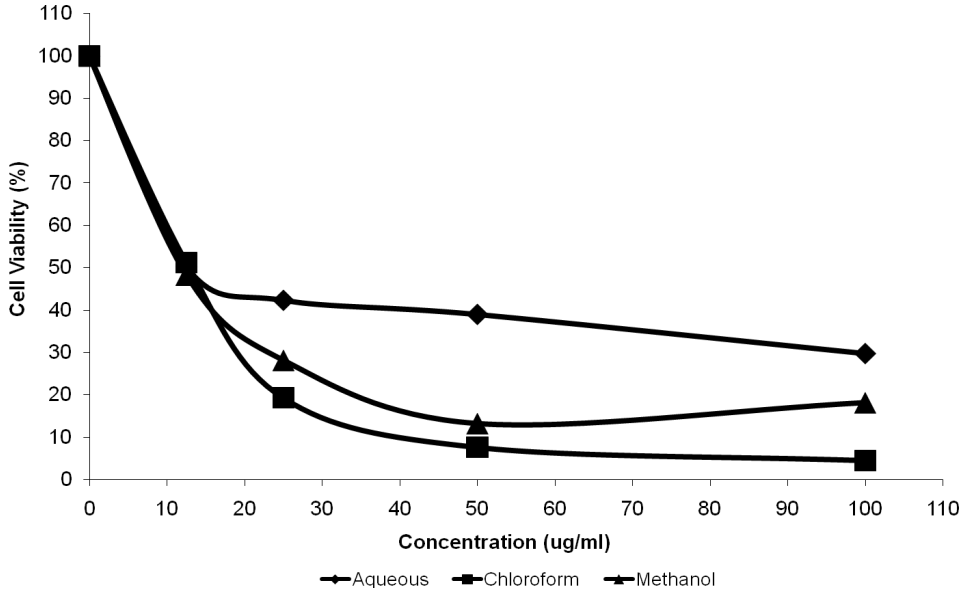

Figure 5. *In vitro* antiproliferative activity of the aqueous, chloroform and methanol extracts of *B. purpurea* leaves against HL-60 cancer cell lines.

methanol extracts of *B. purpurea* leaves to inhibit the proliferation of Caov-3 cancer cell lines is demonstrated in Figure 4. All extracts exhibited antiproliferative activity with the aqueous, followed by chloroform and methanol extracts produced IC_{50} values of 16, 25 and 41 µg/ml, respectively.

Figure 5 shows the antiproliferative profile of the aqueous, chloroform and methanol extracts of *B. purpurea* leaves against the HL-60 cancer cell line. All extracts inhibited the proliferation of HL-60 cells with the methanol, aqueous, and chloroform extracts producing IC_{50} values of 12, 13 and 14 µg/ml, respectively. The antiproliferative

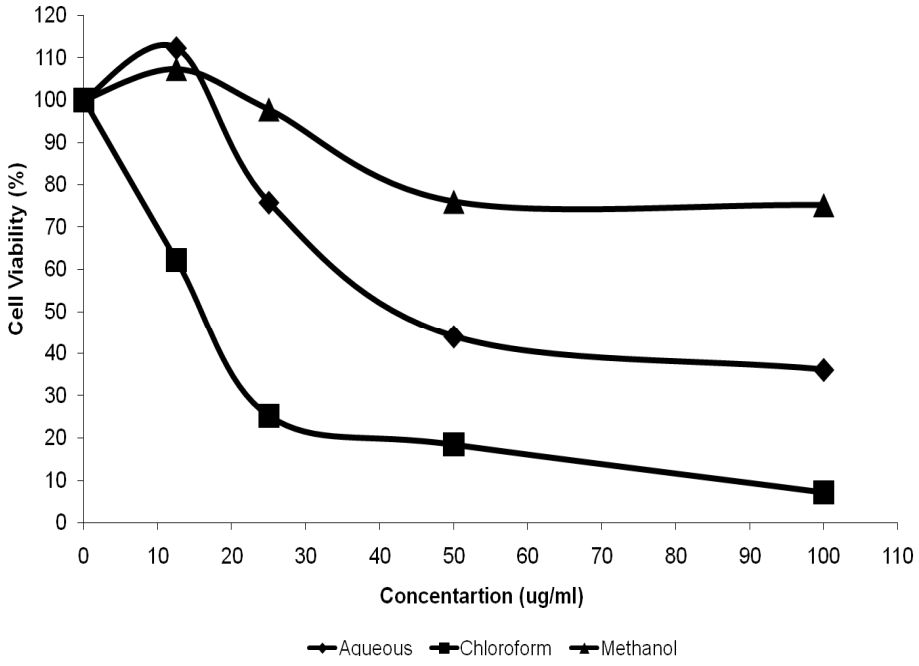

Figure 6. *In vitro* antiproliferative activity of the aqueous, chloroform and methanol extracts of *B. purpurea* leaves against CEMss cancer cell lines.

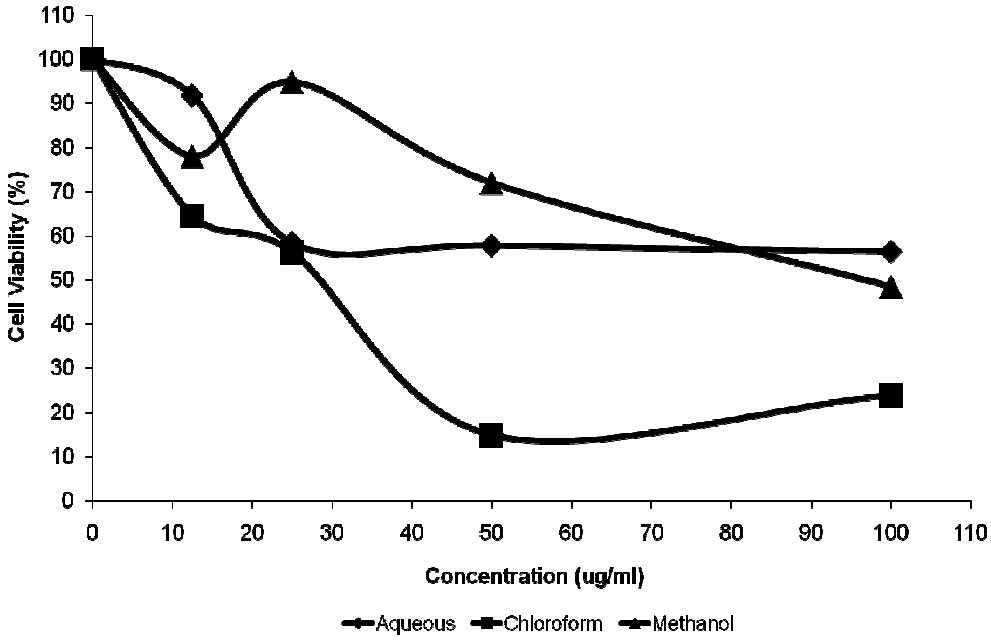

Figure 7. *In vitro* antiproliferative activity of the aqueous, chloroform and methanol extracts of *B. purpurea* leaves against HeLa cancer cell lines.

profile of the aqueous, chloroform and methanol extracts of *B. purpurea* leaves against the CEMss cancer cell line is shown in Figure 6. Only the chloroform and aqueous extracts of *B. purpurea* leaves exhibited antiproliferative activity against CEMss with the IC_{50} values of 18 and 63 µg/ml, respectively.

The effect of aqueous, chloroform and methanol extracts of *B. purpurea* leaves on the proliferative potential of HeLa cancer cell lines can be seen in Figure 7, where the chloroform extract followed by the aqueous and methanol extracts of *B. purpurea* were found to produce IC_{50} values of 21, 24 and 84 µg/ml, respectively.

Table 1. Comparison of the IC_{50} values between tamoxifen and the *B. purpurea* extracts.

Compound/extract		IC_{50} values μmg/ml)						
		3T3	MCF-7	MDA-MB-231	CaOV3	HL-60	CEMss	HeLa
Tamoxifen		ND	13[a]	8 [a]	9 [a]	ND	6 [a]	ND
B. purpurea extracts	Aqueous	ND	9	17	16	12	63	24
	Chloroform	ND	23	19	25	13	18	21
	Methanol	ND	83	75	41	14	ND	84

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 the aqueous, chloroform and methanol extracts of the leaves of *B. purpurea* assessed by DPPH radical scavenging and superoxide scavenging assays.

Sample	Extract	Concentration (μg/ml)	DPPH radical scavenging (%)	Superoxide scavenging (%)
Control	BHT	5	44.3 ± 0.6	-
	Quercetin	20	-	35.7 ± 1.3
B. purpurea	Aqueous	20	0.8 ± 0.4	26.1 ± 3.7
		100	6.1 ± 1.9	31.9 ± 1.5
		500	25.5 ± 0.6	89.4 ± 0.25
	Chloroform	20	0.1 ± 0.1	17.5 ± 0.1
		100	8.6 ± 1.2	31.6 ± 1.8
		500	55.1 ± 1.2	61.6 ± 1.2
	Methanol	20	3.2 ± 0.5	38.2 ± 0.5
		100	15.1 ± 0.2	15.1 ± 0.2
		500	57.7 ± 0.1	57.7± 0.1

Comparison of the antiproliferative activity of those extracts of *B. purpurea* leaves was made against tamoxifen using their IC_{50} values (Table 1). Generally, tamoxifen exhibited potent antiproliferative activity when compared against the respective extract as indicated by its lower IC_{50} values.

Table 2 shows the antioxidant activities of the aqueous, chloroform and methanol extracts of *B. purpurea* assessed using the DPPH radical scavenging and superoxide dismutase scavenging assays. The 20, 100 and 500 μg/ml of aqueous, chloroform and methanol extracts produced low to moderate DPPH radical scavenging activity with 0.7-25.0, 0.1-55.1 and 3.2-57.7%, respectively. The same extracts produced 26.1-89.4, 17.5-61.6 and 14.2-88.1% of superoxide scavenging activity. The superoxide dismutase scavenging activity produced by 500 μg/ml aqueous and methanol extracts was considered high when compared with the chloroform extract.

The total phenolic content (TPC) of the aqueous, chloroform and methanol extracts (2 and 10 mg/ml) were also determined (Table 3). The aqueous extract, at 2 and 10 mg/ml, was found to contain the highest phenolic content, which contains approximately 2571 and 2073 mg/100g of gallic acid, respectively. In addition, the 10 mg/ml methanol extract also contain considerably high total phenolic content (1023 mg/100 g gallic acid). Preliminary screening for phytochemical constituents in the aqueous, chloroform and methanol extracts of *B. purpurea* revealed the presence of different sets of compounds in each of the extract (Table 4). The aqueous extract contained only saponins; the chloroform extract contained flavonoids and steroids; the methanol extract contained saponins, flavonoids and steroids.

DISCUSSION

The present study demonstrated the antiproliferative activity of the aqueous, chloroform and methanol extracts of the leaves of *B. purpurea* against various panel of cancer cell lines. Interestingly, all extracts did not show any cytotoxic effect on the normal cell (3T3) indicating that they are safe. The aqueous and chloroform extracts were effective against all cancer cells with the former exhibiting the lowest IC_{50} value against MCF-7 (≈ 9 μg/ml), MDA-MB 231 (≈ 17 μg/ml) and Caov-3 (≈ 16 μg/ml), while the later was highly effective against the CEMss (≈ 18 μg/ml) and HeLa (≈ 21 μg/ml) cell lines. The methanol extract was highly effective only against the HL-60 (≈ 12 μg/ml).

Table 3. The total phenolic content of the aqueous, chloroform and methanol extracts of the leaves of *B. purpurea*.

Sample	Extract	Concentration (µg/ml)	Total phenolic content (mg/100 g of gallicacid)
B. purpurea	Aqueous	0.2	577.2 ± 0.5
		2	2073.2 ± 0.3*
		10	2570.7 ± 4.9*
	Chloroform	0.2	63.2 ± 6.5
		2	165.7 ± 1.0
		10	405.0 ± 0.2
	Methanol	0.2	220.6 ± 0.1
		2	762.5 ± 9.3
		10	1023.0 ± 1.1*

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/100 g is considered high TPC.

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

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

All extracts were also found to exhibit a low to moderate antioxidant activity when assessed using the DPPH radical scavenging assay. However, the aqueous and methanol extracts showed high antioxidant activity when assessed using the superoxide anion scavenging assay as indicated by the higher percentage of radical scavenging activity (< 80%). Generally, the antioxidant properties of these extracts were found to be concentration-dependent. Based on the results obtained, the aqueous and methanol extracts, which are more polar solvent extracts, were more effective antioxidants compared to the non polar chloroform extract in both assays. This finding is in conformity with the report by Chang et al. (2007).

Studies on medicinal plants/herbs with high phenolic contents have gained importance over the past few years due to the high antioxidant (Zhu et al., 2004), anti-inflammatory (Kim et al., 2004) and anti-carcinogenic activities (Yang et al., 2001), and are of great value in decreasing the risk of many human diseases. Furthermore, flavonoids and other phenolic compounds derived from plants demonstrated scavenging activity and thus, viewed as potential therapeutic drugs for free radical pathologies (Zhu et al., 2004). The antioxidative activities observed can be attributed to either the different mechanisms exhibited by different polyphenolic compounds that is, tocopherols, flavonoids and other organic acids and to the synergistic effects of different compounds. According to Huang et al. (2005), the antioxidant effect of these compounds could be attributed to the different functional properties carried by them, which includes: (1) The reactive oxygen species scavenging activity as seen with quercetin and catechin; (2) inhibition of the generation of free radicals and chain-breaking activity as seen with p-coumaric acids; (3) metal chelation activity. Determination of the total phenolic content showed that. the aqueous- followed by the methanol and chloroform-extracts, contain the highest phenolic compounds. Although previous reports have correlated the high antioxidant activity to the presence of high total phenolic content (Yang et al., 2006), those findings were only in line with the results obtained with the superoxide dismutase scavenging assay, but not with the DPPH scavenging assay. However, it is believed that, the total phenolic contents of these extracts contribute to the low to moderate antioxidant activity when assessed using the latter assay. We have previously reported on the antinociceptive and anti-inflammatory activities of the aqueous extract of *B. purpurea* leaves assessed using various animal models (Zakaria et al., 2007). It is believed that, the free radical scavenging effects took part as one of the mechanisms through which the extracts of *B. purpurea* exhibited their anticancer activity in the present study and anti-inflammatory activity as reported previously (Yang et al., 2006).

Taking into consideration the absence of flavonoid in the aqueous extract despite its high total phenolic content, the *in vitro* inhibition of cancer cell proliferation observed in the present study cannot be solely explained by the concentration of phenolic/flavonoid compounds. Other phytochemicals, particularly saponins and possibly non-flavonoid type of phenolic compounds may play a major role in the antiproliferative activity of the aqueous extracts (Huang et al., 2005; Lemeshko et al., 2006). The presence of flavonoids and saponins are believed to

contribute partly to the antiproliferative activity observed through the antioxidant and free radical scavenging effects.

Based on the bioactive compounds detected in those extracts, several possible antiproliferative mechanisms could be proposed. Saponins have been shown to cause necrotic cell death (Russo et al., 2005) or cell cycle disruption (Roy et al., 2007). In addition, Lee et al. (2006) have confirmed that, the disruption of the cell cycle activity was attained through the inhibition of nuclear factor-kappa B (NF-κB) or through the decreasing number of cells in G0/G1 phase followed by the initial increases in S and G2/M. Other than that, saponins were also reported to induce apoptosis through permeabilization of the mitochondrial membranes (Lemeshko et al., 2006). Flavonoids have been reported to: (1) Induce the Ca^{2+}-dependent apoptotic or the cyclin-dependent kinase inhibitors mechanisms; (2) modulate the cell cycle arrest at the G1/S phase; (3) inhibit the cell survival kinase and the inflammatory transcription factors; or (4) down regulate the anti-apoptotic gene products (Sergeev et al., 2006). In addition, flavonoids also modulate the expression of pro-inflammatory gene of nitric oxide synthase (NOS) and cyclooxygenase-2 (COX-2) (Kim et al., 2004), signifying its capability to modulate the oxidative processes. In conclusion, the present study demonstrated that the *B. purpurea* leaves' extracts possessed significant antiproliferative and antioxidant activities, which are somehow not attributed to their phenolics/flavonoids contents and required further extensive studies.

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