International Journal of Pharmacy and Pharmacology ISSN: 2326-7267 Vol. 9 (2), pp. 001-009, February, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Phenolic content and antioxidant activity of crude and fractionated extracts of *Pereskia bleo* (Kunth) DC. (Cactaceae)

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Accepted 16 September, 2019

The total phenolic content of the extracts of *Pereskia bleo* were assessed by the Folin-Ciocalteau's method while the antioxidant activities were determined by three different assays, namely scavenging activity on 1,1- diphenyl-2 - picrylhydrazyl (DPPH) radicals, reducing power assay and ß-carotene method. In the present study, the ethyl acetate extract exhibited the highest total phenolic content (40.12 mg of GAEs/g of extract) and showed the strongest antioxidant activity in the ß -carotene bleaching assay significantly. Whilst, the hexane extract showed significantly the highest antioxidant activity when determined by scavenging effect on DPPH radicals (EC_{50} 210 µg/ml) and the reducing power assay.

Key words: *Pereskia bleo*, Cactaceae, antioxidant activity, phenolic content.

INTRODUCTION

Ever since the epidemiological consequences of free radicals on certain diseases were disclosed, there is a growing interest in searching for the beneficial health effects of certain foods and plants. Many of the health benefits have been attributed to the antioxidants present in food, beverages and medicinal plants (Lee and Halliwell, 2001). Natural antioxidants are generally pre-sumed to be safe since they occur in plants and are seen as more desirable than their synthetic counterparts.

Peres0kia bleo (Kunth) DC. (Cactaceae), commonly known as 'Jarum Tujuh Bilah' in Malaysia has been used as natural remedy in cancer-related diseases, either eaten raw or taken as a concoction brewed from fresh plant. *P. bleo* is a spiny shrub which can reach up 2 to 8
m (Wahab et al., 2009). The 5 cm orangish-red flowers open late in the afternoon and last only one day. If fertilized, *P. bleo* create a waxy hemispherical yellow fruit that is quite conspicuous. *P. bleo* likes full sun and dry conditions and propagation is by means of cuttings. The plant is believed to have anti-cancer, anti-tumour, anti-rheumatic, anti-ulcer and anti-inflammatory properties. The leaves of the plant are also used as remedy for the relief of headache, gastric pain, ulcers, haemorrhoids and atopic dermatitis; and refresh the body (Goh, 2000; Rahmat, 2004; Tan et al., 2005). The pounded leaves of *P. bleo* paste is also applied to the wound or cut for pain relief. In Panama, locals use the whole plant of *P. bleo* to treat gastrointestinal problems (Gupta et al., 1996).

P. bleo was found to have cytotoxic activity against various cancer cell lines, such as human nasopharyngeal epidermoid carcinoma cell line (KB), human cervical carcinoma cell line (CasKi), human colon carcinoma cell line (HCT 116), human hormone-dependent breast carci-noma cell line (MCF7) and human lung carcinoma cell line (A549); but not toxic to the non-cancer human fibro-blast cell line (MRC-5). These findings were reported in our previous publications (Sri Nurestri et al., 2008, 2009). Although *P. bleo* has no cytotoxic effect on normal cells, it is however reported by Er et al. (2007) that the plant has mutagenic effect. Thus, studies are now conducted to confirm this report.

To our knowledge, there is only one antioxidant study on DPPH radical scavenging activity reported for *P. bleo* (Wahab et al., 2009). However, the extraction method used in their study was not same as in the present study. Various methods have been developed to measure total antioxidant activity, but none have been shown to be

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Abbreviation: BHA, Butylated hydroxyanisole.

Table 1. The preparation of different concentrations of gallic acid solution for calibration plot.

Gallic acid (mg/l)	Gallic acid stock solution (ml)	Methanol (ml)
0	0.000	1.000
25	0.005	0.995
50	0.010	0.990
75	0.015	0.985
100	0.020	0.980
150	0.030	0.970
200	0.040	0.960
250	0.050	0.950
500	0.100	0.900
1000	0.200	0.800

ideal (Erel, 2004). These antioxidant methods detect variable characteristics in the samples. This explains why different antioxidant detection methods lead to different observations. Therefore, usage of different antioxidant methods is necessary in antioxidant activity assessment.

Since development of cancer can be prevented by consumption of medicinal plants with antioxidative properties, it is of interest to investigate whether *P. bleo* posses these properties. The antioxidative properties can be measured by various assays. In the present study, the total phenolic content of the extracts of *P. bleo* was assessed by the Folin-Ciocalteau's method while the antioxidant activities were determined by three different assays, namely scavenging activity on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals, reducing power assay and ß-carotene method.

MATERIALS AND METHODS

Plant sample collection and identification

The fresh leaves of *P. bleo* were collected from Petaling Jaya, Selangor, Malaysia in September 2006. They were identified by Professor Dr. Halijah Ibrahim of Institute of Biological Sciences, Faculty of Science, University of Malaya, Malaysia and a voucher specimen (SN01-06) was deposited at the herbarium of the Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.

Preparation of extracts

The extracts were prepared as previously described (Sri Nurestri et al., 2008). Briefly, the fresh leaves of *P. bleo* (4526.79 g) were washed, dried in the oven and ground to fine powder (752.92 g) using a blender. The dried, ground leaves (752.92 g) were soaked in 100% methanol (1.5 L) for 3 days at room temperature. The solvent-containing extract was then decantered and filtered. The extraction of the ground leaves were further repeated twice with 100% methanol (1.5 L each time).

The filtrate from each extraction was combined and the excess solvent was evaporated under reduced pressure using a rotary evaporator to give a dark green crude methanol extract (79.81 g, 10.60%). The percentage yield of the crude methanol extract was based on the weight of dried and ground plant materials. The methanol extract (33.58 g) was further extracted with hexane to give a hexane-soluble fraction (2.19 g, 6.52%) and a hexane insoluble residue.

The hexane-insoluble residue was further partitioned between ethyl acetate–water (1: 1, 100: 100 ml) to give an ethyl acetate-soluble extract (0.99 g, 2.95%). The water layer was freeze-dried to give a brown coloured water extract (18.14 g, 54.02%). The percentage yield of fractionated extracts was based on the weight of the crude methanol extract. The weights of all the extracts were measured after solvent evaporation. All the extracts were kept in the dark at 4°C for not more than one week prior to evaluation of antioxidant activities and total phenolic content.

Determination of total phenolic content

The concentrations of phenolic compounds in the extracts of *P. bleo*, expressed as gallic acid equivalents (GAEs), were measured according to the Folin-Ciocalteu method described by Cheung et al. (2003) and Singleton et al. (1965).

Preparation of gallic acid calibration plot

Briefly, a calibration plot, using gallic acid with concentrations ranging from 25 to 1000 mg/l was prepared. Gallic acid stock solutions (Sigma-Aldrich) in volumes ranging from 0.005 to 0.2 ml were pipetted out into test tubes. The final volume was made to 1 ml with methanol in each test tube (Table 1). Different concentrations of the resultant gallic acid solution (0.02 ml) and negative control (methanol was used instead of gallic acid) were mixed with 1.58 ml of distilled water. Folin-Ciocalteu's phenol reagent (0.1 ml) was added to each test tube.

After 3 min, 0.3 ml of saturated sodium carbonate (Na_2CO_3) solution (~35 %) was added to the mixture. The reaction mixtures were incubated at 40°C for 30 min. The blank contained only methanol. The absorbance was determined at 765 nm with a spectrophotometer. The gallic acid calibration plot was obtained by plotting the absorbance against concentration of gallic acid (mg/l). Table 2 summarizes the preparation of reaction mixture for phenolic content determination.

Determination of total phenolic content in test extracts and positive reference standard

BHA (butylated hydroxyanisole) was used as positive reference

Table 2. The preparation of reaction mixture for phenolic content determination.

Reagent	Volume (ml)
Gallic acid solution from Table 1	0.02
Distilled water	1.58
Folin-Ciocalteu's phenol reagent	0.10
Saturated sodium carbonate (Na_2CO_3) solution	0.30
Total volume	2.00

standard in the study. All the test extracts and BHA standard were prepared at concentration of 20 mg/ml in methanol as stock extracts.

The extracts (or BHA standard) (0.02 ml) at different concentrations (4, 8, 12, 16 and 20 mg/ml) and control (methanol was used instead of extract) were mixed with 1.58 ml of distilled water. Folin-Ciocalteu's phenol reagent (0.1 ml) was then added to each test tube. After 3 min, 0.3 ml of saturated sodium carbonate (Na_2CO_3) solution (~35%) was added to the mixture. The reaction mixtures were incubated at 40°C for 30 min. The blank contained only methanol. The absorbance was determined at 765 nm with a spectrophotometer. The blank contained only methanol. All extracts were assayed in triplicate.

Scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals

The scavenging activity of the extracts of *P. bleo* on DPPH radicals was measured according to the method of Cheung et al. (2003). All samples were dissolved in methanol and assayed in triplicate. Ascorbic acid and BHA were used as positive reference standards. The scavenging activity (%) on DPPH radicals was calculated according to the following equation:

$$\text{Scavenging activity (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100\%$$

where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the extract/standard.

The scavenging ability of the extracts was expressed as EC_{50} value, which is the effective concentration at which 50% of DPPH radicals were scavenged. The EC_{50} value was obtained from the graph of scavenging activity (%) versus concentration of samples. Low EC_{50} value indicates strong ability of the extract to act as DPPH scavenger. The higher the EC_{50} value indicates the lower the scavenging activity of the scavengers as more amount of the scavengers were required to achieve 50% scavenging reaction and thus the scavengers are less effective in scavenging the DPPH radicals.

Reducing power assay

The reducing power of the prepared extracts was determined according to method of Oyaizu (1986). All samples were assayed in triplicate. Increased absorbance of the reaction mixture indicates greater reducing power. Mean values from three independent samples were calculated for each extract. Ascorbic acid and BHA were used as positive reference standards. The higher absorbance of the reaction mixture indicates greater reducing power. The extracts that showed comparable absorbance readings with ascorbic acid and BHA are considered having high reducing power.

ß-Carotene bleaching method

The antioxidant activity of the prepared extracts was determined according to the ß-carotene bleaching method described by Cheung et al. (2003). All samples were assayed in triplicate. BHA was used as standard. The rate of ß-carotene bleaching (R) was calculated according to equation as below:

$$R = [\ln (A_0/A_t)] \setminus t$$

where ln is natural logarithm, A_0 is absorbance at time 0, A_t is absorbance at time t, and t is 20, 40, 60, 80, 100 or 120 min.

The antioxidant activity (%) was calculated in terms of percentage inhibition relative to the control using the equation below:

$$\text{Antioxidant activity (\%)} = \left(\frac{R_{control} - R_{sample}}{R_{control}} \right) \times 100\ \%$$

Statistical analysis

The antioxidant data were subjected to one-way analysis of variance (ANOVA) and the significance of the difference between the means was determined by the Duncan's multiple range tests at 95% least significant difference ($p < 0.05$).

RESULTS AND DISCUSSION

Total phenolic content of *P. bleo* extracts

Phenols are one of the major groups of non- essential dietary components that have been associated with the inhibition of atherosclerosis and cancer, as well as for age-related degenerative brain disorders (Cheung et al., 2003; Wang et al., 2009). They can range from simple molecules, such as phenolic acids, to highly polymerized compounds, such as tannins. According to previous reports, a highly positive relationship between total phenols and antioxidant activity is found in many plant species (Gulcin et al., 2004).

The Folin-Ciocalteau reagent-based assay is commonly used to determine the total phenolic content. The total phenolic contents in the *P. bleo* extracts, determined from regression quotation of calibration curve, were expressed as mg of GAEs per gram of methanol or dry fractionated extracts as usually reported by earlier researchers. The total phenolic content [mg/l of gallic acid equivalents (GAEs)] was obtained using the following formula which was automatically derived from the gallic acid calibration plot:

$$\text{Total phenolic content (mg/l of GAEs)} = \frac{(y - 0.0004)}{0.0012}$$

Where y is the absorbance value of the test extract and BHA after subtraction of control.

Table 3. Concentration of total phenolics of *P. bleo* extracts.

Extracts	Weight of extracts (g)	Concentration of total phenolics (mg of GAEs/g of methanol or fractionated dry extracts)	Yield of phenolics (g of GAEs)	Percentage of yield of phenolics (%)
Methanol	79.81	27.88 ± 0.28 [a]	2.23	2.79
Hexane	2.19	23.15 ± 0.35 [a]	0.05	2.28
Ethyl acetate	0.99	40.12 ± 0.66 [b]	0.04	4.04
Water	18.14	27.70 ± 0.18 [a]	0.50	2.76
BHA*	-	252.97 ± 2.81	-	

* Positive reference standard; GAEs, gallic acid equivalents; Values expressed are mean ± standard deviation of three measurements; Means with different letters in the same column are significantly different ($p < 0.05$, ANOVA).

The concentration of total phenolics of *P. bleo* extracts are shown in Table 3. The total phenolic content in the ethyl acetate extract was highest at 40.12 mg of GAEs/g of extract, although the yield of ethyl acetate extract was the lowest among the fractionated extracts (refer to 'Preparation of extracts' in 'Materials and Methods'). The methanol, hexane and water extracts had similar amount of phenolic content, which were 27.88, 23.15 and 27.70 mg of GAEs/g of extract, respectively. The high phenolic content of ethyl acetate extract may have contributed towards its antioxidant and related activity.

Two phenolic compounds, 2,4-di- tert-butylphenol and -tocopherol, reported present in the ethyl acetate extract (Sri Nurestri et al., 2009), may have contributed to the total phenolic content of the ethyl acetate extract. 2, 4-Di-tert-butylphenol is structurally related to the well known antioxidant BHA (butylated hydroxyanisole). Yoon et al. (2006) reported that 2,4-di-tert-butylphenol exhibited antioxidant activities on copper-mediated oxidation (IC_{50} value of 8.2 µM), AAPH-mediated oxidation (IC_{50} value of 9.9 µM) and SIN-1 mediated oxidation (52%) in the TBARS assay. -Tocopherol, reported to be capable of eliminating free radical damage, was regarded as intracellular antioxidants and found to protect cells from carcinogenic chemicals due to its ability in inhibiting the peroxidation of polyunsaturated fatty acids and its damaging free-radical-mediated consequences (Jiang et al., 2001; Bermudez et al., 2007; Choi and Lee, 2009). It is therefore highly probable that these two phenolic compounds may have contributed to its high phenolic content and antioxidant and related activities.

Scavenging activity of *P. bleo* extracts on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals

Free radical scavenging is one of the known mechanisms by which antioxidants inhibit cellular damage. The DPPH free radicals scavenging method is a colorimetric assay and can be used to evaluate the radical scavenging capacity of specific compounds or extract in a short time (Cheung et al., 2003). DPPH is a nitrogen-centred free radical, stable at room temperature and produces a purple solution in methanol. In its radical form, DPPH shows an absorbance maximum at 520 nm which disappears upon reduction by an antioxidant. This is visualized as a discolouration from purple to yellow in a spectrophotometer (Bondet et al., 1997; Duh et al., 1999; Chang et al., 2002; Gulcin et al., 2004).

P. bleo contained active substances, including phenolic compounds (Table 3). The fast electron-transfer from the phenoxide anions of the phenolic compounds to the DPPH radicals is a possible mechanism for their reducing capacity (Huang et al., 2005). Kadoma et al. (2009) and Yoon et al. (2006) reported that 2, 4-di-tert-butylphenol showed strong radical scavenging activity in the DPPH assay with IC_{20} value of 1.01 mM.

In the DPPH scavenging assay, extracts of *P. bleo* were investigated through the free radical scavenging activity *via* their reaction with the stable DPPH radicals (Figures 1 and 2). The radical scavenging activity (EC_{50} values) of extracts and positive reference standards on DPPH radicals are shown in Table 4.

Generally, in comparison to both positive reference standards, ascorbic acid (EC_{50} 19 µg/ml) and BHA (EC_{50} 11 µg/ml), all the extracts displayed moderate scavenging activity, except the water extract which exhibited the weakest scavenging effect (EC_{50} 1.70 mg/ml; Table 4 and Figure 1) . Among the extracts, the hexane extract was the best scavenger with the lowest EC_{50} value (210 µg/ml), followed by ethyl acetate, methanol and water. The results were consistent with those of Wahab et al. (2009). The result here revealed that extracts of *P. bleo* can be considered as free radical inhibitors or scavengers.

Reducing power of *P. bleo* extracts

Many studies have indicated that antioxidant effect is related to the development of reductones as reductones were reported to be terminators of free radical chain reactions (Dorman et al., 2003; Shon et al., 2003; Duan et al., 2006) . Thus, the antioxidant activity of *P. bleo* extracts may relate to their reducing ability.

In the reducing power assay, the yellow colour of the test

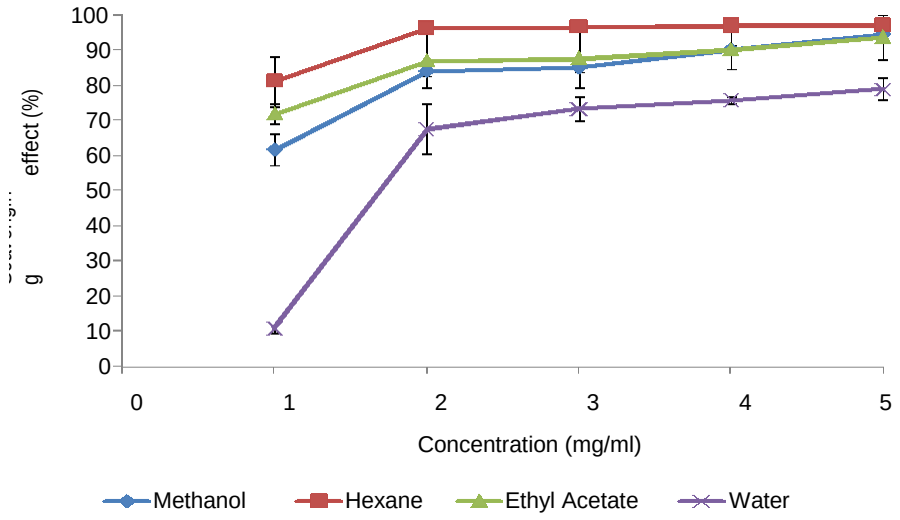

Figure 1. Scavenging effect of *P. bleo* extracts on DPPH radical. Each value is expressed as mean ± standard deviation of three measurements.

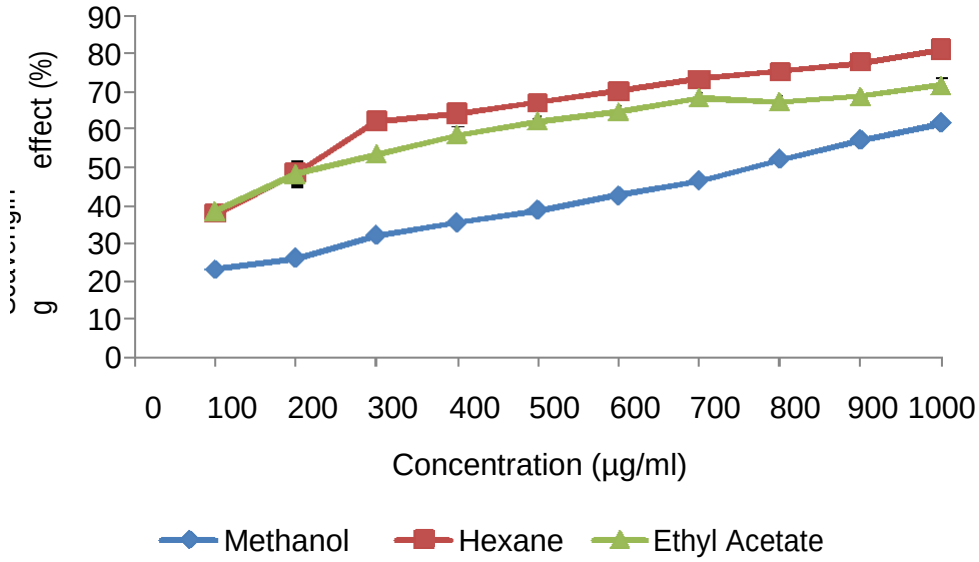

Figure 2. Scavenging effect of lower concentrations of *P. bleo* extracts on DPPH radicals to determine the EC_{50} values. Each value is expressed as mean ± standard deviation of three measurements.

Table 4. The scavenging activity (EC_{50} values) of *P. bleo* extracts on DPPH radicals.

Extracts	EC_{50} values (µg/ml)
Methanol	750
Hexane	210
Ethyl acetate	225
Water	1700
Ascorbic acid*	19
BHA*	11

*Positive reference standard; EC_{50}, 50% effective concentration.

solutions change to various shades of green and blue depending upon the reducing power of each extract. The presence of reductants (that is, antioxidants) in the test extracts result in the reduction of the Fe^{3+} / ferric cyanide complex to the ferrous form (Fe^{2+}). The Fe^{2+} ions can therefore be monitored by measuring the formation of Pearl's Prussian blue at 700 nm. Increased absorbance at 700 nm indicates an increase in reducing power of test extracts (Lih et al., 2001). The reducing power of *P. bleo* extracts and positive reference standards are shown in Table 5 and Figure 3.

As shown in Table 5 and Figure 3, the reducing power

Table 5. Reducing powers of *P. bleo* extracts at various concentrations.

Extracts	Concentrations of extracts (mg/ml)			
	5	10	15	20
Methanol	0.645 ± 0.03 ^{w}	1.100 ± 0.05 ^{x}	1.465 ± 0.03 ^{x}	1.605 ± 0.04 ^{x}
Hexane	1.633 ± 0.02 ^{x}	1.846 ± 0.03 ^{y}	2.072 ± 0.06 ^{z}	2.222 ± 0.04 ^{z}
Ethyl acetate	0.665 ± 0.00 ^{w}	0.890 ± 0.07 ^{w}	1.009 ± 0.00 ^{w}	1.155 ± 0.05 ^{w}
Water	0.670 ± 0.06 ^{w}	1.175 ± 0.02 ^{x}	1.676 ± 0.05 ^{y}	1.834 ± 0.03 ^{y}
Ascorbic acid*	2.343 ± 0.05	2.451 ± 0.02	2.496 ± 0.02	2.579 ± 0.04
BHA*	2.432 ± 0.01	2.458 ± 0.03	2.549 ± 0.02	2.616 ± 0.02

*Positive reference standard; Absorbance values expressed are mean ± standard deviation of triplicate measurements. For different extracts with the same concentration, means in the same column with different letters (w-z) were significantly different ($p < 0.05$, ANOVA).

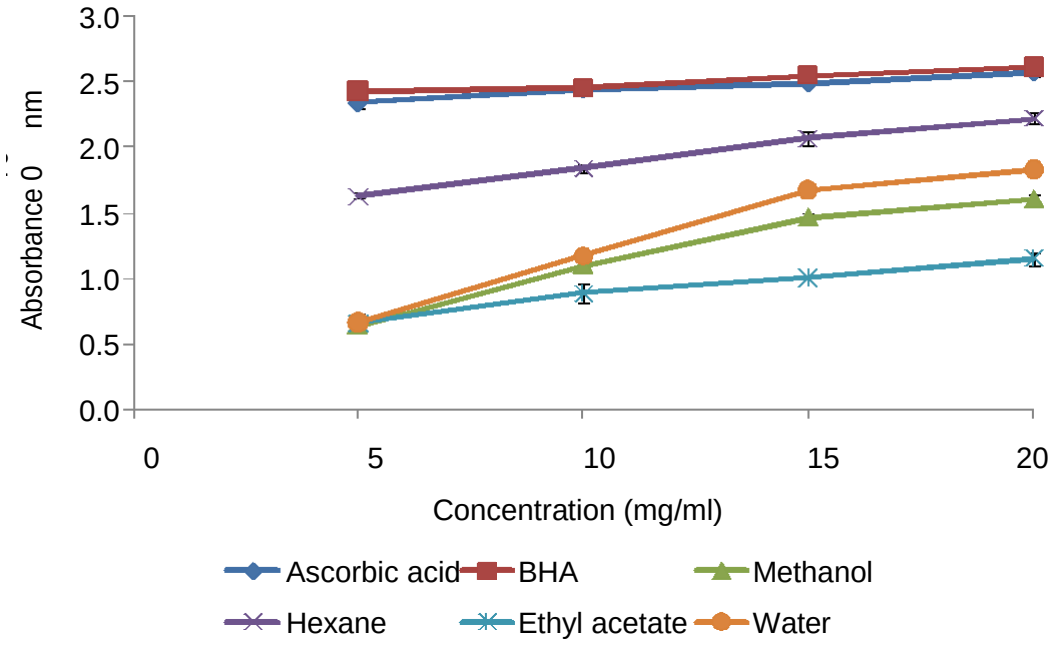

Figure 3. Reducing powers of extracts of *P. bleo* at various concentrations. Each value is expressed as mean ± standard deviation of three measurements.

of all the extracts gradually increased with increasing concentration of the extracts. This indicated that all extracts possessed the ability (either strong or weak) to reduce Fe^{3+}/ ferric cyanide complex to the ferrous form when being evaluated by reducing power assay. However, the reducing power of the positive reference standards (ascorbic acid and BHA) were relatively more pronounced than the tested extracts. Ascorbic acid and BHA showed high reducing power which indicated high UV absorption (2.343 - 2.616) at wavelength 700 nm. The reducing powers of ascorbic acid and BHA at 5 mg/ml were 2.343 and 2.432, respectively and increased to 2.579 and 2.616, respectively at 20 mg/ml.

The reducing power of all tested extracts varied significantly with different concentrations ($p < 0.05$; Table 5). The hexane extract showed significantly ($p < 0.05$) the highest reducing activity among the extracts of *P. bleo* with 1.633 at 5 mg/ml, 1.846 at 10 mg/ml and 2.072 at 15 mg/ml and 2.222 at 20 mg/ml (Table 5). The results presented revealed that the hexane extract has the highest electron donating capacity possibly contained higher amounts of reductones.

The water and methanol extracts possessed some considerable reducing activity compared to the positive reference standards while the ethyl acetate extract showed the lowest ($p < 0.05$) reducing power (Table 5 and Figure 3). The hexane extract of *P. bleo* was found to contain mainly methyl esters, such as methyl palmitate, methyl linoleate, methyl -linolenate and phytol which were identified by GCMS analysis. It is highly probable that the lone pairs of electron on the carbonyl oxygen of the fatty esters can be easily donated to the ferric ions in

Table 6. Antioxidant activity (%) of *P. bleo* extracts measured by ß-carotene bleaching method.

Extracts	Concentrations of extracts (mg/ml)			
	4	8	16	20
Methanol	34.62 ± 0.98 ^{x}	37.90 ± 0.62 ^{w}	64.03 ± 2.96 ^{xy}	72.96 ± 2.78 ^{x}
Hexane	40.57 ± 1.15 ^{xy}	46.11 ± 1.17 ^{x}	62.38 ± 4.19 ^{x}	73.31 ± 1.60 ^{x}
Ethyl acetate	42.21 ± 4.05 ^{y}	52.87 ± 4.71 ^{y}	69.92 ± 3.22 ^{y}	83.68 ± 0.57 ^{y}
Water	27.97 ± 5.24 ^{w}	36.84 ± 2.45 ^{w}	48.38 ± 2.27 ^{w}	46.17 ± 1.56 ^{w}
BHA*	70.80 ±1.09	79.00 ± 0.52	88.56 ± 0.82	92.46 ± 2.52

*Positive reference standard; Values expressed are mean ± standard deviation of triplicate measurements. For different extracts with the same concentration, means in the same column with different letters (w - z) were significantly different ($p < 0.05$, ANOVA).

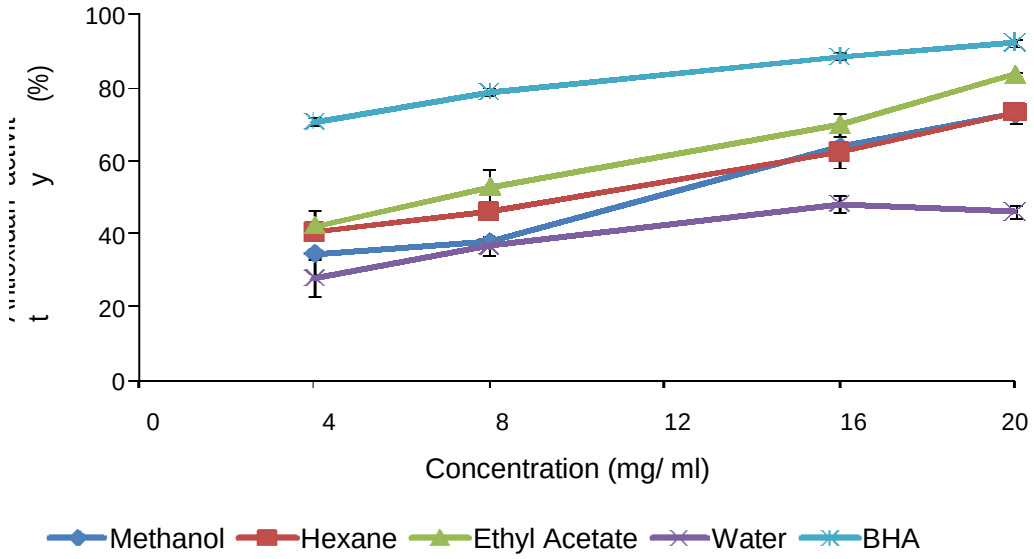

Figure 4. Antioxidant activity (%) of *P. bleo* extracts measured by ß-carotene bleaching method. Each value is expressed as mean ± standard deviation of three measurements.

in the reducing power assay.

ß-Carotene bleaching activity of *P. bleo* extracts

Bleaching of ß-carotene is a free-radical-mediated phenomenon resulting from the hydroperoxides formed from linoleic acid by air oxidation. In the absence of antioxidants, the ß-carotene molecules lose their double bonds by oxidation as well as the characteristic orange colour, which can be monitored spectrophotometrically. The presence of different antioxidants can hinder the extent of ß-carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system (Jayaprakasha et al., 2001) . Thus, this forms the basis by which *P. bleo* extracts can be screened for their antioxi-dant potential. Table 6 and Figure 4 show the antioxidant activities of the *P. bleo* extracts and BHA with the coupled oxidation of ß-carotene and linoleic acid. The antioxidant activities of the extracts varied significantly with different concentrations ($p < 0.05$; Table 6).

The antioxidant activities of all the extracts gradually increased with increasing concentration of the extracts (Figure 4). Among the *P. bleo* extracts, the water extract showed significantly ($p < 0.05$) the weakest antioxidant activity while the ethyl acetate extract presented significantly ($p < 0.05$) the strongest activity (at all concentrations tested).

The ethyl acetate extract exhibited 83.68% antioxidant activity respectively at 20 mg/ml, which was comparable to that of BHA standard at 20 mg/ml (92.46%; Table 6) . It is highly probable that the antioxidative components in the ethyl acetate extract can reduce the extent of ß-carotene destruction by neutralizing the linoleate free radical and other free radicals formed in the system. This indicated that compounds with the strongest antioxidant activity in the ß -carotene-linoleate assay system were of medium polarity.

Conclusions

The combination of four methods applied in this study gave valuable information in the evaluation of the antioxidant activity of *P. bleo* extracts and could be recommended for other similar investigations.

In the present study, the ethyl acetate extract exhibited the highest total phenolic content and showed the highest antioxidant activity in the ß-carotene bleaching assay significantly ($p < 0.05$). On the other hand, the hexane extract of *P. bleo* showed significantly ($p < 0.05$) the highest antioxidant activity when determined by scavenging effect on DPPH radicals (at concentrations of extracts >1mg/ml) and reducing power assay. It is thus suggested that the antioxidant activities of the hexane extract were not solely contributed by phenolic compounds but other antioxidants in the extracts. Some of the antioxidant compounds which appeared in the ethyl acetate extracts might also appear in the hexane extracts or *vice versa*. However, more methods such as the lipid peroxidation and the thiobarbituric acid-malondialdehyde inhibitory activity need to be performed to establish the potential activity of the ethyl acetate extract in comparison to standard positive controls.

2, 4-Di-tert-butylphenol, -tocopherol, ß-sitosterol and a mixture of sterols (campesterol, stigmasterol and -sitosterol), were isolated and identified from the ethyl acetate extract of *P. bleo* (Sri Nurestri et al., 2009). These compounds were reported to possess antioxidant activities (Rensburg et al., 2000; Jiang et al., 2001; Wang et al., 2002; Homma et al., 2003; Yoon et al., 2006; Bermudez et al., 2007; Choi and Lee, 2009; Kadoma et al., 2009). Thus, it is suggested that the antioxidant activity of the ethyl acetate extract may be partly contributed by the above compounds while 2, 4-di-tert-butylphenol and -tocopherol contributed to the phenolic content. However, the synergism among the antioxidants in the extract made the antioxidant activity, not only dependent on the concentration of antioxidants, but also on the structure and interaction among the antioxidants (Sun and Ho, 2005).

In conclusion, the present study confirms that *P. bleo* can be a significant source of natural antioxidant that may have potent beneficial health effects.

ACKNOWLEDGEMENTS

This work was supported by research fund from the University of Malaya (PPP PS241/2008C) and Fundamental Research Grant Scheme from Ministry of Higher Education (FP040/2008C). We are also grateful to Prof A. Hamid A. Hadi for use of his laboratory space.

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