Antioxidant activity of different extracts from leaves of Pereskia bleo (Cactaceae)

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The present study was conducted to assess the antioxidant properties of ethanol, ethyl acetate, methanol and hexane extracts from leaves of Pereskia bleo using 2,2-diphenyl-1-picrylhydrazyl method (DPPH assay), ferric reducing antioxidant power (FRAP assay) and β-carotene-linoleic acid methods. Total phenolic compounds (TPC) and flavonoids of the leaves of P. bleo were measured using Folin-Ciocalteu and HPLC methods respectively. Results of the present study showed that Ethyl acetate extract of P. bleo leaf exhibited significantly (p < 0.05) higher antioxidant activity as measured using DPPH free radical (IC₅₀), FRAP and β-carotene-linoleic assays than that of hexane extract, methanol extract and ethanol extract. Pereskia bleo leaf was also found to contain high amounts of bioactive compounds including total phenolic compounds (109.43 ±0.84 mg GAE/g), epicatechin (575± 0.04 mg/100g), quercetin (110.94 ±0.12 mg/100g), catechin (918 ± 0.01 mg/100 g) and myricetin (9.49±0.55 mg/100g) while the concentrations of α-tocopherol, β-carotene and lycopene were found to be higher (69±0.25, 51.97±1.18 mg/100 g, 92.46±0.41 µg/g), respectively.

Key words: Pereskia bleo, antioxidant activity, total phenolic compounds, total flavonoid compounds.

INTRODUCTION

Oxidative stress can cause a wide range of diseases including Alzheimer's and Parkinson's disease, diabetes, cardiovascular disease, rheumatoid arthritis, cancer and other diseases (Simonian, 1996). Antioxidants are compounds that are capable of slowing or preventing the oxidation of other molecules. Antioxidants can terminate chain reactions by removing free radicals and oxidation reaction blocking others by being oxidized themselves. Due to this, antioxidants such as polyphenols and flavones that are often reducing factors (Shahidi, 1998).

Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are very effective in neutralizing free radicals and therefore they are used as inhibitors of lipid peroxidation to stabilize food containing fat. Carcinogenicity and toxicity of these synthetic antioxidants have been reported (Shahidi, 1997; Jeong et al., 2004, Siddhuraju and Becker, 2003). Consequently, the use of synthetic antioxidants is limited. In recent years, researchers are trying to replace them with natural antioxidants such as tocopherols, flavonoids and rosemary for food preservation (Bruni et al., 2004; Frutos and Hernandez-Herrero, 2005; Hras et al., 2000; Williams et al., 2004).

Fruits and vegetables are reported to contain large amount of antioxidants, so people who consume these products have a lower risk of the relevance of free radical diseases such as heart disease and neurological disorders. Moreover, medicinal plants have been shown to have antioxidant activity by virtue of the presence of phenolic diterpenes, flavonoids, tannins and phenolic acids (Dawidowicz et al., 2006). Medicinal plants have been regarded as a source of bioactive natural compounds. Antioxidants have the ability to protect the body against damages caused by oxidative stress.

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induced free radicals. There is a growing interest in natural antioxidants, for example, polyphenols present in medicinal plants and food materials, which could help to prevent oxidative damages (Silva et al., 2005).

Plant phenolic compounds probably had multifunctional antioxidants. These compounds have been reported to quench free radicals derived from oxygen, giving a hydrogen atom or an electron to the radical (Wanasundara and Shahidi, 1998). The antioxidant properties of phenolic compounds have been demonstrated in many systems through studies in vitro and in human low-density lipoproteins and liposomes (Leanderson et al., 1997). Literature showed only one antioxidant study on DPPH radical scavenging activity reported for *P. bleo* (Malek et al., 2008). However, the extraction method used in their study was different from that used in the present study. Various methods have been developed to measure total antioxidant activity, but none have been shown to be ideal (Erel, 2004). These antioxidant methods detect variable characteristics in the samples. This explains why different antioxidant detection methods may lead to different observations. Consequently, 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* possess these properties. In the present study, the total phenolic compound of the extracts of *P. bleo* was assessed by the Folin-Ciocalteau’s method while the antioxidant activities were determined using three different assays, namely scavenging activity on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals, reducing power assay and β-carotene method.

In Malaysia, herbs are usually eaten fresh as a vegetable (salad), especially among the Malay communities (Frankel and Meyer, 2000). Locally known as the ‘Seven Star Needle’ (qi xing zhen), this plant is a member of the cactus family. The genus was named in honour of Nicolas Fabre de Peiresc, a French botanist of the 16th Century (Figure 1).

However, this cactus is a leafy cactus that is not a desert-adapted plant like many other leafless cacti. The objective of this study was to investigate the antioxidant compounds and its activities of extracts from *P. bleo* leaves using different solvent systems.

### MATERIALS AND METHODS

#### Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tri-pyrdidyl-s-triazine (TPTZ) were obtained from Sigma-Aldrich Company(USA), β-carotene, linoleic acid, Tween 40, 2,6-di-tert-butyl-4-hydroxytoluene (BHA), 2,6-di-tert-butyl-4-methylphenol (BHT) and 2,4,6-Tris(2-pyrdidyl)-s-triazine (TPTZ), 6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and Folin-Ciocalteu phenol reagent were also obtained from Sigma (St.Louis, MO, USA). Methanol of HPLC-grade and hexane obtained from Merck was used for extractions. All other chemicals used were of analytical grade.

#### Collection of plant material

*P. bleo* leaves were collected from the vicinity of Universiti Putra Malaysia (UPM) in October 2009. They were identified by Professor Dr. Norihan Saleh of Faculty of Biotechnology and Biomolecular Science, University Putra Malaysia. Voucher specimen (CA01-09) was deposited in the Department of Food Biotechnology lab, Faculty of Food Science and Technology, UPM, Malaysia.

#### Preparation of plant extracts

Fresh leaves of *P. bleo* were harvested and all petioles were cut and removed. The leaves were washed and dried under oven (Memmert, Germany). The dried leaves were ground in a grinder (Sharp,Malaysia). The dried powder was stored in the dark glass bottle at 4ºC. After filtration with whatman No1 filter paper using vacuum pump, the samples were concentrated using a rotary vacuum evaporator (EYELA, USA) at 40°C to dryness. The concentrated extracts were kept in dark bottle at 4ºC.

#### Determination of antioxidant activity assays

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activity of *P. bleo* was performed using the method of DPPH radical scavenging activity as described by Brand-Williams et al. (1997). This method is spectrophotometric assay using a stable radical 2, 2′-diphenylpicrylhydrazyl (DPPH) as a reagent (Bondent and Brand-Williams, 1997; Cuendet et al., 1997; Buritis and Bucar, 2000). Different concentration of sample was (0.5ml) added to 3.5 ml of methanol DPPH solution with 6× 10⁻⁵ mol/L, concentration in test tubes and kept covered at room temperature for 30 min. After the incubation period, absorbances were read at 515 nm until the reaction reached a plateau. The percentage of

**Figure 1.** *Pereskia bleo* shrub.
radical scavenging activity was calculated as follows:

$$\text{Inhibition\%} = \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100$$

$$A_{\text{blank}} = \text{Absorption of blank sample (t = 0 min)}; \quad A_{\text{sample}} = \text{Absorption of tested extract solution (t = 30 min)}.$$  Extract concentration providing 50% inhibition ($IC_{50}$) was calculated from the plot of percentage inhibition against concentration of the extract. Hydroxanisol (BHA) and α-tocopherol were used as positive controls. All assays were carried out in triplicate.

### Ferric reducing antioxidant potential assay

Determination of FRAP was performed using the method described by Benzie and Strain (1996) with slight modifications. The stock solutions contains 300 mM acetate buffer (3.1 g C$_2$H$_5$NaO$_2$, 3H$_2$O and 16 ml C$_2$H$_4$O$_2$), pH 3.6, 10 mM TPTZ solution in 40 mM HCl and 20 mM FeCl$_3$, 6H$_2$O solution. The stock solution (FRAP reagent) was prepared by mixing 50 ml acetate buffer, 5 ml TPTZ, and 5 ml FeCl$_3$, 6H$_2$O solution (ratio 10:1:0.1). Two hundred microliters of sample was added to 3 ml of FRAP reagent. After incubation in dark for 30 min at 37°C and the absorbance was read at 593 nm (Shimadzu UV-1650 PC UV-Vis spectrophotometer). Final results were expressed as micromole Trolox equivalent (TE) per gram of extract basis (μmol TE/g). The amounts of the value of FRAP show the effects of antioxidant activity in the sample.

### β-carotene- linoleic acid assay

In the assay system acid β-carotene-linoleic acid, an aqueous emulsion of β-carotene and linoleic acid under condition of heat-induced oxidation reaction is used for testing anti-oxidant activity. This test is a rapid staining of β-carotene in the absence of antioxidant (Miller, 1971). In this process, the linoleate free radicals formed during the reaction are neutralized by antioxidants.

Briefly, 200 mg linoleic acid (Sigma, 99%) and 20 mg of Tween 20 (Sigma) were placed in a flask, then 2 mg of β-carotene (Sigma, 95% purity) dissolved in 10 ml chloroform (Merck, Germany) was added. Chloroform was evaporated using a vacuum evaporator unit. Then 50 ml of distilled water saturated with oxygen by stirring for 30 min was added. Aliquots (200 ft) of each sample dissolved in methanol (2.0 mg/ml) were added to 2.5 ml of solution in test tubes. The samples were then placed in an oven at temperature of 50°C for 3 h. The absorbance was read at 470 nm using a spectrophotometer. Percent antioxidant activity was calculated from the following equation:

$$\text{AA\%} = 100 \times \left( 1 - \frac{A_{\text{O}} - A_{\text{T}}}{A_{\text{AO}} - A_{\text{A}}} \right)$$

Where AA% is percent of antioxidant activity, $A_{\text{O}}$ is the absorbance at beginning of reaction, $A_{\text{T}}$ is the absorbance after three hour, with compound; $A_{\text{AO}}$ is the absorbance at beginning of reaction, without compound (200 μl of methanol and 2.5 ml of stock solution). $A_{\text{A}}$ is the absorbance after 3 h without compound. A White stock solution containing less β-carotene has been used to bring the spectrophotometer to zero. As a positive control 2, 6-di-tet-butyl-4-methylphenol (BHT, Sigma) was used with same condition and concentration (2.0 mg/ml). Each test was repeated three times and the average score and standard deviation calculated.

### Determination of total phenolic compound (TPC)

TPC was measured by colorimetry using the Folin-Ciocalteu (Sigleton et al., 1999). One ml of appropriate dilution (using 80% ethanol) ethanol extract of leaves $P$. bleo was added to a 25 ml volumetric flask filled with 9 ml of distilled water. Phenol reagent of Folin-Ciocalteu (0.5 ml) and 5 ml of 5% Na$_2$CO$_3$ were added to the sample and shake vigorously. The solution was immediately make up to 25 ml with distilled water and mix well. The incubation time was one hour at room temperature under dark condition. The absorbance was then measured at 765 nm. Quantification was based on the calibration curve of gallic acid standard, and the contents were expressed as mg gallic acid equivalents (mg)/100 g.

### Statistical analysis

Data were analyzed using statistical package for social sciences (SPSS), version 16. Difference between means was determined using ANOVA (Duncan and Tukey’s test).

### RESULTS AND DISCUSSION

#### 1.1- Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay

DPPH method measured the ability of antioxidant in scavenging free radicals present. Antioxidant activity of $P$. bleo leaf was expressed as the concentration that inhibits 50% DPPH free radical ($IC_{50}$). Results obtained in the study showed that the $IC_{50}$ of ethyl acetate extract of $P$. bleo (168.35 ± 6.5 μg/ml) was significantly (p < 0.05) lower than that of other extracts; Hexane extract (244 ± 4.5 μg/ml), methanol extract (277.5 ± 6.5 μg/ml), and ethanol extract (540 88 ± 9.0 μg/ml (Figure 2).

In this study, the ethyl acetate extract of leaves of $P$. bleo was found to exhibit excellent antioxidant activity compared to that of ethanol, hexane, and methanol extracts. The results are compatible with those of Wahab et al. (2008) and Sri Nurestri et al. (2009). All extracts showed low activity against BHA and-tocopherol, which act as a reference antioxidant compounds. There was broad experimental evidence suggesting that being a rich source of polyphenolic compounds, plants display strong antioxidant activity (Sabu et al., 2002).

#### FRAP method

The FRAP test measures the ability of samples to reduce ferric ion to the ferrous form of TPTZ (2,4,6-tripryridylstrijazine). One FRAP unit is defined as the reduction of 1 mol of Fe$^{3+}$ to Fe$^{2+}$). Similarly, result of the study showed that the antioxidant capacity of ethyl acetate extract (50.48 ±1.53 μM TE/g) was significantly (p < 0.05) higher than that of hexane (41.7± 2.2 μM TE/g) methanol and ethanol extracts (45.2±1.2 μM TE/g), (40.4±1.54 μM TE/g), respectively, of leaves of $P$. bleo (Figure 3).

However, there was no significant (p < 0.05) difference on the antioxidant capacity between hexane extract and methanol extract. It is interesting to note that the trend of antioxidant activity obtained from FRAP assay was similar to that obtained in DPPH assay.
The antioxidant activity ability of the plant extracts basically depend on the composition of the extracts, hydrophobic or hydrophilic nature of the antioxidants, type of solvent used for extraction process, method of extraction, temperature and conditions of the test systems. Therefore, it is necessary to use more than one method for evaluation of antioxidant activity of plant extracts defining various mechanisms of antioxidant actions (Wong et al., 2006). Antioxidant capacity and health benefits of medicinal plants are often ascribed to their total phenolic contents (Hua-Bin, 2008; Nagai et al., 2003). High correlation was also reported between antioxidant activity as measured by DPPH method and total phenolic compound and flavonoid content of 21 selected tropical plants (Mustapha et al., 2010). Similarly, green tea exhibited higher total antioxidant activity than that of pure catechins proportionately combined based on the green tea constituent, which is mainly due to its synergistic effect (Rice-Evans et al., 1995) (Table 1).
Antioxidant activity of different extracts of Pereskia bleo leaves using linoleic acid-β-Carotene method.

Table 1. Determination of DPPH, FRAP and β-Carotene-linoleic scavenging activity using of Pereskia bleo in different extracts.

<table>
<thead>
<tr>
<th>Samples</th>
<th>DPPH IC₅₀(mg/ml)</th>
<th>FRAP (µM TE/g extract)</th>
<th>β-carotene-linoleic acid(AA%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α- tocopherol*</td>
<td>27.13 ± 0.7ᵇ</td>
<td>54.91±1.52ᵇ</td>
<td>78±0.34%ᵇ</td>
</tr>
<tr>
<td>BHA*</td>
<td>11.75±2.5ᵃ</td>
<td>77.75±2.19ᵃ</td>
<td>84.5±0.21%ᵃ</td>
</tr>
<tr>
<td>Hexane Extract(leaf)</td>
<td>244±4.5ᵈ</td>
<td>41.7±2.2²,ᵈ</td>
<td>66±1.2%ᵈ</td>
</tr>
<tr>
<td>Methanol Extract (leaf)</td>
<td>277.5 ± 6.5ᵉ</td>
<td>45.2±1.2ᶜ</td>
<td>67.8±4.6%ᵈ</td>
</tr>
<tr>
<td>Ethanol Extract(leaf)</td>
<td>540.88 ± 9.0¹</td>
<td>40.45±1.54ᵈ</td>
<td>65±2.7%ᵈ</td>
</tr>
<tr>
<td>Ethyl Acetate Extract (leaf)</td>
<td>168.35 ± 6.5ⁱ</td>
<td>50.48±1.53ᵇ</td>
<td>71±4.5%ᵉ</td>
</tr>
</tbody>
</table>

*= control positive values with different letters are significantly different (P<0.05) according to Duncan Test, SPSS Version 16.

Bioactive compounds in P. bleo leaves

Selected bioactive compounds from P. bleo leaf were determined and showed in Table 2. Results of the study showed that TPC of P. bleo leaf were 109.43 ± 0.84 mg GAE/g of extract. The major flavonoids identified were catechin (918 ± 0.01 mg/100 g) and quercetin (110.94 ± 0.12 mg/100 g). This was followed by epicatechin (109.43 ± 0.84 mg/100 g) and myricetin (9.49 ± 0.55 mg/100 g). However, concentrations of β-carotene (51.97 ± 1.18 mg/100 g) and α- tocopherol (69 ± 0.25 mg/100 g) were quite high.

Conclusion

The results of experiments in vitro, including radical scavenging DPPH, FRAP assay, determining the content of phenolic and total β-carotene bleaching showed that phytochemicals in extracts of plants studied can have a significant effect on antioxidant activity. In addition, the antioxidant activity is directly related to the total amount of phenolic compounds in plant extracts. These compounds have been reported to possess antioxidant activities. P. bleo can be used as an easily accessible source of natural antioxidants, and can be used as food supplement or in pharmaceutical and medical industries.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Ministry of
Table 2. Bioactive compound in dried leaves of *Pereskia bleo*.

<table>
<thead>
<tr>
<th>Samples</th>
<th>mg/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechin</td>
<td>918±0.01b</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>575±0.04c</td>
</tr>
<tr>
<td>Quercetin</td>
<td>110.94±0.12d</td>
</tr>
<tr>
<td>Myricetin</td>
<td>9.49± 0.55³</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>51.97± 1.18³</td>
</tr>
<tr>
<td>Tocopherol</td>
<td>69± 0.25⁹</td>
</tr>
<tr>
<td>Total phenolic contents</td>
<td>10943±0.84³</td>
</tr>
</tbody>
</table>

Data represents mean ± standard deviation. Means followed by the different alphabets are significantly (p <0.05) different (n=3). *=Value based on mg GAE/100 g.

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REFERENCES


