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Antioxidant properties of different cultivars of *Portulaca oleracea*

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Abstract

Methanolic extracts of six cultivars of *Portulaca oleracea* were analyzed for their total phenol content (TPC) using the Folin–Ciocalteu method. The antioxidant activity was measured using the 1,1-diphenyl-2-picrylhydrazyl, ferric-reducing antioxidant power (FRAP) and β-carotene bleaching (BCB) assays. The iodine titration method was used to determine the ascorbic acid content (AAC). The TPC of the cultivars of *P. oleracea* ranged from 127 ± 13 to 478 ± 45 mg GAE/100 g of fresh weight of plant. There was good correlation between the TPC value and its AEAC, IC_{50} and FRAP values ($r^2 > 0.9$) for all the cultivars. The AAC for the cultivars ranged from 38.5 ± 0.6 to 73.0 ± 17.5 mg/100 g. The TPC value of the common variety PO1, was the lowest compared to the ornamental cultivars (PO2–PO6). The BCB assay showed that all cultivars were capable of inhibiting lipid peroxidation and the inhibition power did not correlate with TPC value.

Keywords: *Portulaca oleracea*; Total phenol content; DPPH; IC_{50}; Ferric-reducing antioxidant power; β-Carotene bleaching

1. Introduction

There are about 70 species of edible herbs in Malaysia, which are called by their local name *ulam* (Samy, Sugumaran, & Lee, 2004). Some of these herbs are claimed to have high antioxidant properties as well as medicinal properties. The complex mixture of phytochemicals in vegetables and fruits provide overlapping or complementary effects that contribute to the protective effect of health (Chu, Sun, Wu, & Liu, 2002). Several studies have shown a positive correlation between the total content of phenolic compounds and the antioxidant activity in plants (Benzie & Szeto, 1999; Katalinic, Milos, Kulisic, & Jukic, 2006; Lugasi & Hovari, 2003). These herbs have additional properties like anti-inflammatory effects, anti-bacterial and anti-fungal effects (Kahkonen et al., 1999).

Portulaca oleracea (Portulacaceae family) is listed in the World Health Organization as one of the most used medicinal plants and it has been given the term 'Global Panacea' (Dweck, 2001; Samy et al., 2004). The stems and leaves of the plant are succulent and edible with a slightly acidic and salty taste similar to spinach. It is available commercially in both ornamental and culinary cultivars. Widely used as a potherb in the Mediterranean, Central European and Asian countries, it is also referred to as the common Purslane. The aerial parts of the plant are used medicinally for alleviating pain and swelling, and as an antiseptic (Chan et al., 2000). The dried herb can be boiled and is made into tea/soups in China (Cai, Luo, Sun, & Corke, 2004).

Recent research has shown that the *P. oleracea* is a rich source of omega-3 fatty acids, which is important in preventing heart attacks and strengthening the immune system (Simopoulos, 2004). It was reported to contain gallotannins (Lewis, 2003), kaempferol, quercetin and apigenin (Cai et al., 2004; Radhakrishnan et al., 2001). The water extracts of *P. oleracea* show no cytotoxicity or genotoxicity, and have been certified safe for daily consumption as a vegetable (Yen, Chen, & Peng, 2001). This plant was reported to have neuropharmacological actions, wound healing activities and bronchodilatory effects (Malek, Boskabady, Borushaki, & Tohidi, 2004; Parry, Marks, & Okwuasaba, 1993; Rashed, Afifi, & Disi, 2003). Dietary glutathione, normally occurring in high amounts in fresh

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meat and in moderate amounts in some fruits and vegetables, is found in the *P. oleracea* (Simopoulos, 2004). The methanol extracts from this plant were found to exhibit moderate antimicrobial activity against *Bacillus subtilis* (Sakai, Inada, Okamoto, Shizuri, & Fukuyama, 1996). The inhibitory effect on lipopolysaccharide (LPS) and interferon-γ (IFN-γ)-induced NO production was shown by the extracts of *P. oleracea* in a concentration dependent manner (Abas, Lajis, Israf, Khozirah, & Kalsom, 2006).

In spite of extensive studies made on the bioactivities of this plant, the antioxidant properties of *P. oleracea* have rarely been studied. The influence of the different cultivars on the total phenol content and its antioxidant activity has not been reported. This paper aims to fill in the knowledge gap for this useful plant.

2. Materials and methods

2.1. Chemicals and reagents

Arsenic trioxide, gallic acid, Folin–Ciocalteu's phenol reagent, linoleic acid and Tween-40 were obtained from Fluka (Switzerland). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), quercetin and β-carotene were obtained from Sigma (St. Louis, MO). Potassium dihydrogen phosphate, di-potassium hydrogen phosphate trihydrate and ascorbic acid were obtained from Merck (Germany). Solvents used were from Fisher Chemicals (Springfield, NJ).

2.2. Plant materials

Some of the plants were obtained from a nursery in Sungai Buluh and some from the author's garden. The plants were harvested during or prior to their flowering period. Analysis was carried out on the same day as the collection of the plant. The plant material was identified morphologically as *P. oleracea* using proper references to the literature (Boo, Omar-Hor, & Ou-Yang, 2003). The six varieties studied were arbitrarily labeled as PO1–PO6 (Fig. 1). PO1, known as Gelang pasir in Malaysia and Indonesia, is a vegetable (Samy et al., 2004).

2.3. Sample preparation and extraction

The parts of the plant suitable for consumption were used, which consisted of the soft upper stems of the plant and leaves. The discolored and insect-damaged portions were discarded. At the initial stage, three different solvents (methanol, ethanol and water) for extraction of the plant material (PO5 was used) were used to determine the solvent that has the highest extraction efficiency.

Two grams of the plant were weighed, added with liquid nitrogen and ground into fine powder with mortar and pestle. One hundred milliliters of solvent was added to the finely ground plant and the mixture was swirled in an orbital shaker for 1 h. To test for extraction efficiency of each solvent, second and third extractions were made by transferring the residue from the first extraction into another flask and adding 100 ml of solvent. The extracts were filtered and stored in a −20 °C freezer. For a given batch, all analyses were completed within two weeks.

2.4. Ascorbic acid content (AAC)

AAC was determined by the iodine titration method, which was modified from a procedure by Silva, Simoni, Collins, and Volpe (1999). As_2O_3 was used to standardize iodine/potassium iodide solution. For some samples a RP–HPLC procedure was used: mobile phase 5% acetic acid; C-18 column (Waters Symmetry; 3.9 × 150 mm; 5 μm) and monitoring wavelength 254 nm. Both methods gave similar results within experimental error.

2.5. Determination of total phenol content (TPC)

Total phenol contents were determined using a method developed by Singleton and Rossi (1965). One and a half milliliters of Folin–Ciocalteu's reagent (diluted 10 times) and 1.2 ml of Na_2CO_3 (7.5% w/v) solution were added to 300 μl of plant extract. Mixtures were shaken and left to stand at room temperature for 30 min before measuring absorbance at 765 nm using a spectrophotometer (Anthelie Advanced 5 Secoman, France). The determination was done in triplicate. The total phenol content (TPC) was expressed as gallic acid equivalent in mg/100 g fresh plant material.

As ascorbic acid content (AAC) in plant interferes with the quantification of total phenol content (TPC), corrections have been made for all the TPC values in this study by subtracting the AAC from the total phenol value using an ascorbic acid standard curve.

2.6. 1,1-Diphenyl-2-picrylhydrazyl free radical scavenging activity

The ability of a compound to donate a hydrogen atom was assessed on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical according to a procedure based on Miliauskas, Venskutonis, and van Beek (2004) with slight modifications. Two milliliters of 0.15 mM DPPH was added to 1 ml of extracts in different dilutions. A control was prepared by adding 2 ml of DPPH to 1 ml of methanol. The contents of the tubes were mixed and allowed to stand for 30 min, and absorbance was measured at 517 nm. Triplicate tubes were prepared for each extract. The results were expressed as % radical scavenging activity.

$$\%\ \text{radical scavenging activity} = (1 - A_{\text{sample}}/A_{\text{control}}) \times 100$$

IC_{50} which denotes the amount (mg) of plant in 1 ml solution required to reduce initial concentration of DPPH radicals by 50% was also calculated. Ascorbic acid was used as a standard and results were expressed in ascorbic acid equivalent antioxidant activity (AEAC) using the equation:

Variety 1 (PO1)	Variety 2 (PO2)	Variety 3 (PO3)
		
Yellow flower ; single – layered petals ; paddle – shaped leaves ; red stem	Yellow flower ; Single-layered petals ; wedge – shaped leaves ; green stem	White flower ; single layered petals ; wedge –shaped leaves ; green stem

Variety 4 (PO4)	Variety 5 (PO5)	Variety 6 (PO6)
		
Orange-red flower; single-layered petals ; wedge – shaped leaves ; red stem	White – pink flower ; single – layered petals ; wedge – shaped leaves ; green stem	Yellow flower ; single – layered petals ; wedge-shaped leaves ; red stem

Fig. 1. The different cultivars of *Portulaca oleracea*.

AEAC (mg AA/100 g) = $IC_{50(ascorbate)}/IC_{50(sample)} \times 100{,}000$

2.7. Ferric-reducing antioxidant power (FRAP)

This method was proposed by Oyaizu (1986) which involves the presence of antioxidants in extract to reduce the ferricyanide complex to the ferrous form. One milliliter of extracts in different dilutions were added to 2.5 ml phosphate buffer (0.1 M, pH 6.6) and 2.5 ml potassium ferricyanide (1% w/v). The mixture was then incubated in a water bath at 50 °C for 20 min followed by 2.5 ml trichloroacetic acid (10% w/v) solution. The contents of the tubes were mixed well and 2.5 ml of solution was removed from each tube. To this, 2.5 ml solution, 2.5 ml water and 0.5 ml ferric chloride solution (0.1% w/v) were added. The mixtures were allowed to stand for 30 min before absorbance measurements were taken at 700 nm. Triplicate tubes were prepared for each extract. The FRAP values, expressed in mg GAE/g, were derived from a standard curve.

2.8. β-Carotene bleaching (BCB) assay

The procedure used for evaluating the antioxidant activity of the samples was modified from a method described by Kumazawa et al. (2002). Forty milligrams of linoleic acid and 400 mg of Tween 40 were weighed and added to 3 ml of β-carotene (5 mg/50 mL chloroform) to create a β-carotene/linoleic acid emulsion. Fresh emulsions were prepared for each assay. The chloroform was evaporated off with nitrogen gas. One hundred milliliters of oxygenated water was added to the dry mixture and mixed well. The initial absorbance was taken at time zero.

Ten, 50 and 100 μl of extracts were added to 3 ml aliquots of β-carotene/linoleic acid emulsion. The mixtures were incubated at 50 °C for 60 min. Absorbance at 470 nm and 700 nm was measured after 60 min. Measurements at

700 nm are needed to correct for the presence of haze in the solution. Water instead of plant extract was used as control. Quercetin was used as a standard. The degradation rate (DR) was calculated with the formula:

Degradation rate (DR) of β-carotene

$$= \ln(A_{initial} - A_{sample})/60$$

The antioxidant activity (AOA) was calculated using this formula:

Antioxidant activity (% AOA)

$$= [(DR_{control} - DR_{sample})/DR_{control}] \times 100$$

3. Results and discussion

3.1. Plant specimens

Six different cultivars of *P. oleracea* were analyzed (Fig. 1). Varieties 2, 3, 4, 5 and 6 are the ornamental cultivars with larger flowers that come in shades of red, yellow, orange and white. Variety 1 is the common *P. oleracea* consumed as a vegetable.

3.2. Extraction using different solvents

Water, 100% methanol and 50% ethanol extracts of the plant (two batches; variety PO5) were examined to determine the best extraction solvent to be used in this study. Among the three solvents, the methanol extract yielded the highest TPC (295 mg GAE/100 g fresh weight, based on two samplings). The TPC values of the 50% ethanol extracts and water extracts were lower, being 172 and 141 mg GAE/100 g, respectively. The water extract exhibited a slightly viscous texture which made it unsuitable for study.

Methanol is the most suitable solvent in the extraction in polyphenolic compounds from plant tissue, due to its ability to inhibit the action of polyphenol oxidase that causes the oxidation of polyphenols and its ease of evaporation compared to water (Yao et al., 2004). Methanol extracts have been used in the study of *P. oleracea* flavonoids and some aspects of its antioxidants activities (Abas et al., 2006; Sakai et al., 1996). These results seem contradictory to a study made by Cai et al. (2004) whereby water extraction of a powder sample of *P. oleracea* at 80 °C for 20 min yielded higher total phenol content (0.60 g/100 g DW) than a methanol extraction (0.44 g/100 g DW). The reason could be due to the hydrolysis of the glycosidic and ester bonds of the condensed flavonoids and hydrolysable tannins at near boiling temperature (Oboh, 2005).

To determine the extraction efficiency of the methanol solvent, three extractions were carried out by reusing the residue from the first extraction for the second and third extractions. It was found that methanol effectively extracted all the extractable polyphenols from the plant in the first extraction.

3.3. Variation between plant batches and locations

Four different batches of variety PO5 was collected on different days between September to December 2005 and on different environmental conditions (Table 1). Batch 1 was collected after a week of dry weather (304 ± 9 mg GAE/100 g), batches 2 and 3 during fair weather (223 ± 2 and 227 ± 2 mg GAE/100 g) and batch 4 after rain (157 ± 2 mg GAE/100 g). It was noticed that batch 1 has the highest TPC but the lowest AAC, while batch 4 has the lowest TPC. The variability could be due to environmental factors and collection period. Previous studies have indicated that the level of active compounds in plants increased when sunlight and temperature increased (Ben-Hammouda, Kremer, Minor, & Sarvar, 1995; Bravo, 1998; Corrigan & Sandberg, 2001; Daniel, Meier, Schlatter, & Frischknecht, 1999; Dragland, Senoo, Wake, Holte, & Blomhoff, 2003).

Batches 2, 3 and 4 have higher AAC than batch 1. Degradation of ascorbic acid also depends on several factors like oxygen, metal ion catalyst, light, temperature and moisture content. The study done by Erenturk, Gulaboglu, and Gultekin (2005) on degradation of ascorbic acid in Rosehips (*rosa canina*) showed that ascorbic acid content is higher when moisture content is high and ascorbic acid is rapidly degraded when moisture content is reduced. The IC_{50}values varied between 1.19 ± 0.03 to 1.72 ± 0.05 mg/ml. The AEAC and FRAP values varied between 223 ± 6 to 321 ± 8 mg AA/100 g and 2.00 ± 0.32 to 3.60 ± 0.09 mg GAE/g, respectively.

To check for the variation in locations, variety PO1 was used to conduct this study because it was easily available in two different locations, which was the author's garden in Damansara Utama and Monash University in Sunway. The result shows that there was no significant difference between them. The TPC for the two different places were 121 ± 11 and 132 ± 11 mg GAE/100 g, respectively. The differences in AAC in the two different locations were also not significant with the values of 63.2 ± 12.7 and 68.6 ± 5.8 mg/100 g.

Table 1
Contents of ascorbic acid, total phenols and antioxidant activities in batches of *Portulaca oleracea* variety 5 (PO5) collected on different days

Collection date (weather condition)	Batch	TPC (mg GAE/100 g)	AAC (mg/100 g)	IC_{50} (mg/ml)	AEAC (mg AA/100 g)	FRAP (mg GAE/g)
20th September 2004 (dry)	Batch 1	304 ± 9	53 ± 10	1.19 ± 0.03	321 ± 8	3.60 ± 0.09
4th October 2005 (fair)	Batch 2	277 ± 2	73 ± 5	1.31 ± 0.20	292 ± 46	3.40 ± 0.05
16th October 2005 (fair)	Batch 3	223 ± 2	85 ± 2	1.44 ± 0.13	265 ± 24	3.30 ± 0.18
2nd December 2005 (after rain)	Batch 4	157 ± 2	71 ± 2	1.72 ± 0.05	223 ± 6	2.00 ± 0.32

3.4. Different plant growth stages

This study was conducted to determine mainly the TPC content of the edible (aerial) parts of the plant at different growth stages (Table 2). Only PO6 was chosen for this experiment as it has relatively high antioxidant properties compared to the other *P. oleracea* cultivars.

The young shoots (3–4 weeks old) collected had light green stems and leaves. The developing plant (2–3 months old) was collected before or during the flowering of the plant. At this stage the leaves were dark green and its stem was reddish in colour instead of green. At the mature stage (4–5 months old), the plant was wilting and dry.

The TPC value for the young shoots was significantly lower than that of developing and the mature plant. The TPC at the mature plant stage is slightly lower than the plant at the developing stage. The AAC does not show a significant decrease from the developing to the mature stage of the plant.

A higher level of TPC in the developing leaves could be because protective compounds such as antioxidants are essential at early growth stages. Plants at this stage are more metabolically active as they require higher concentration of essential compounds for growth. The lower TPC values in the mature plant could be due to oxidative stress as the plant is dying off (Witzell, Gref, & Nasholm, 2003).

Components in leaf tissues can also change with maturity; for example phenolic content decreases while anthocyanins and other flavonoids increase. Young leaves from berry crops have higher polyphenols compounds compared to older leaves (Wang & Lin, 2000). Del Rio et al. (2004) studied the level of hesperidin in the different stages of *Citrus limon* growth. It increased from the first formation of immature fruit and reached its maximum at the developing stage, and the level decreased as the fruit grew to maturity state. It can be said that the highest accumulation rate was related to the young stages of development due to intense cellular division. The levels of polyphenolic compounds then decrease rapidly with age due to their dilution in leaf growth (Del Bano et al., 2003).

Table 2
TPC and AAC of young, developing and mature plant for *Portulaca oleracea* variety 6 (PO6)

Plant stage	Age of plant	TPC (mg GAE/100 g)	AAC (mg/100 g)
Young shoots	3–4 weeks	150 ± 6	Not determined
Developing	2–3 months	496 ± 34	74.4 ± 9.7
Mature	4–5 months	397 ± 8	68.2 ± 3.8

3.5. Comparative study among the different cultivars of P. oleracea

Table 3 shows the AAC, TPC, IC_{50}, AEAC and FRAP of six cultivars of *P. oleracea*. The TPC of various cultivars were in the range of 127 ± 13 to 478 ± 45 mg GAE/100 g of fresh weight. The order of TPC among different cultivars is as follows: PO6 > PO5 ≈ PO4 > PO3 > PO2 ⩾ PO1. Based on the TPC, the six cultivars studied can be classified into three groups, namely high level, average level and low level. PO1, PO2 and PO3 were grouped as cultivars with generally low TPC. PO4 and PO5 were grouped together as cultivars with average TPC. PO6 is the cultivar with high TPC. The common variety PO1 has the lowest TPC value, and the highest TPC value is from the ornamental variety PO6. The variety PO6 with high antioxidant properties may provide a source of new antioxidant in diets as well as genes for new improved varieties for use in food and medicinal purposes.

The IC_{50} ranged from 0.89 ± 0.07 to 3.41 ± 0.41 mg/ml, the AEAC values ranged from 110 ± 14 to 430 ± 32 mg AA/100 g and the FRAP values from 0.93 ± 0.22 to 5.10 ± 0.56 mg GAE/g. The higher TPC value corresponded with higher AEAC and FRAP values and lower IC_{50} values. For example, good correlation ($r^2 = 0.9540$) between TPC of different cultivars and AEAC was observed (Fig. 2). A similar relationship was also observed

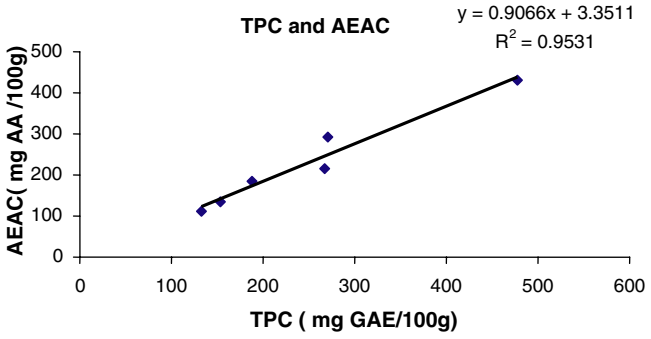

Fig. 2. Correlation between TPC and AEAC in *Portulaca oleracea* cultivars.

Table 3
Total phenol and ascorbic acid contents, DPPH free radical scavenging activity (AEAC and IC_{50}) and ferric-reducing antioxidant power in cultivars of *Portulaca oleracea*

Cultivar	AAC (mg/100 g)	AEAC (mg AA/100 g)	Contribution of AAC to TAA (%)	TPC (mg GAE/100 g)	IC_{50} (mg/ml)	FRAP (mg GAE/g)
PO1	66.4 ± 10.3	110 ± 14	60.4	127 ± 13	3.41 ± 0.41	0.93 ± 0.22
PO2	38.5 ± 0.6	135 ± 35	28.5	153 ± 18	3.04 ± 0.79	1.25 ± 0.50
PO3	55.4 ± 5.7	185 ± 6	29.9	188 ± 10	2.07 ± 0.06	1.60 ± 0.10
PO4	55.8 ± 11.0	215 ± 8	26.0	268 ± 32	1.78 ± 0.07	Not determined
PO5	73.0 ± 17.5	293 ± 29	24.9	271 ± 42	1.31 ± 0.13	3.43 ± 0.15
PO6	68.7 ± 13.8	430 ± 32	16.0	478 ± 45	0.89 ± 0.07	5.10 ± 0.56

in IC_{50} and FRAP which highly correlated with TPC of the different cultivars ($r^2 = 0.9581$; and $r^2 = 0.9592$, respectively) (Figs. 3 and 4).

In terms of contribution of ascorbic acid to the total antioxidant activity (TAA), PO1 with the lowest TPC value of 127 ± 13 mg GAE/100 g has 60.4% of its total antioxidant activity contributed by ascorbic acid while PO6 with the highest TPC value of 478 ± 45 mg GAE/100 g has only 16.0%. This concludes that (except PO1) most of the AOA is contributed by the relatively high phenolic content. The contribution of AAC to the total AOA decreases when the TPC increases.

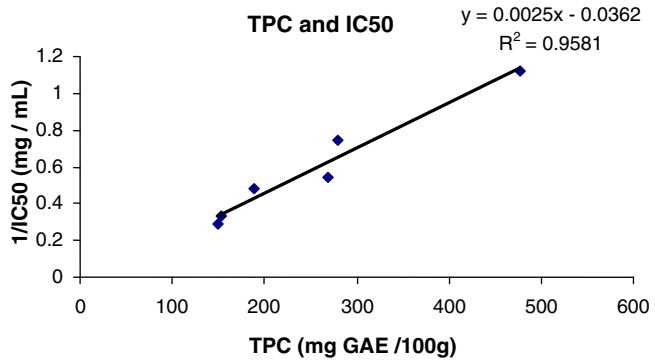

Fig. 3. Correlation between TPC and IC_{50} in *Portulaca oleracea* cultivars.

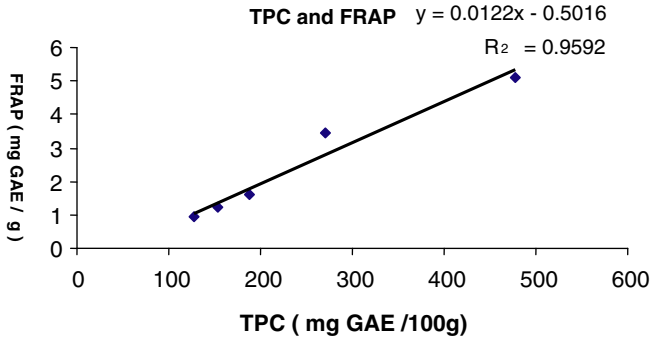

Fig. 4. Correlation between TPC and FRAP in *Portulaca oleracea* cultivars.

All cultivars were capable of inhibiting lipid peroxidation to a certain extent. Fig. 5 shows that the BCB activity is concentration dependent. The inhibiting activity of the extracts was compared with a quercetin standard. In four out of five varieties studied, 1–2 mg of plants (except PO2) extracted into 100 μl methanol have activity comparable to 1–10 μg quercetin. There was no correlation between TPC and antioxidant activity measured by the BCB assay. For example, in spite of much lower TPC for PO1 (TPC = 127 mg GAE/100 g), it has activity similar to that of PO6 (TPC = 478 mg GAE/100 g). The apparent contradiction may be explained in terms of the difference in the relative amounts of the lipophilic antioxidants in the plants (Gadow, Joubert, & Hansmann, 1997). The lipophilic antioxidant molecules partition more into the oily droplets of β-carotene and linoleic acid than the hydrophilic ones. The result implies that PO1 most probably has similar amounts of lipophilic antioxidants as PO6. The results in the BCB assay cannot be correlated to those from the DPPH radical scavenging assay or the FRAP assay because of the different reaction system. Complex composition of extracts could be responsible for certain interactions (synergistic, additive or antagonistic effects) between their components or the medium.

4. Conclusion

In this study, it is found that the ornamental cultivars have higher TPC and antioxidant activities than the common *Portulaca olerecea* (PO1), which is a vegetable plant. In particular, the cultivar PO6 (yellow flower, red and soft succulent stems) has very high TPC and antioxidant activities which were assessed by the 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric-reducing antioxidant power (FRAP) and β-carotene bleaching (BCB) assays. These ornamental cultivars may provide a new source of antioxidants as well as genes and for use in food and medicinal purposes. The TPC correlates well with the antioxidant activities as measured by the DPPH and FRAP assays but does not correlate with BCB activity. The ascorbic acid content (ACC) also varies according to the different cultivars but does not correlate with the antioxidant activities.

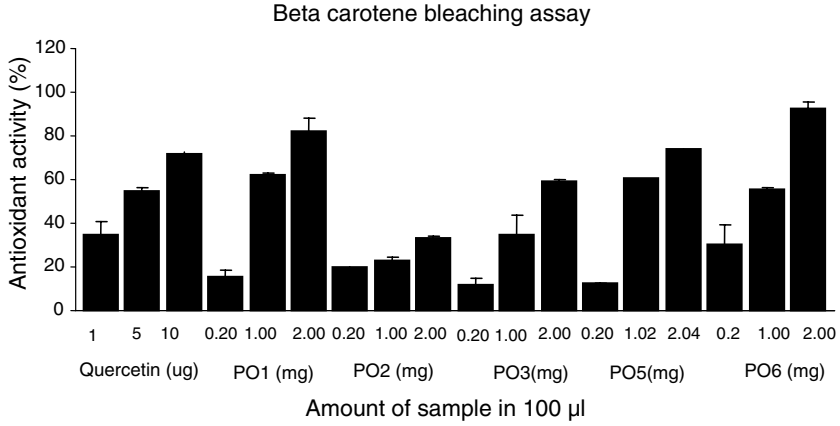

Fig. 5. BCB antioxidant activity of various *Portulaca oleracea* cultivars.

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