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Original Article

Antioxidant Activities of the Methanol Extracts of Various Parts of *Phalaenopsis* Orchids with White, Yellow, and Purple Flowers

Hoang Chinh NGUYEN[1a], Kuan-Hung LIN[2b], Meng-Yuan HUANG[2*], Chi-Ming YANG[3], Tin-Han SHIH[3], Tung-Chuan HSIUNG[2], Yen-Chang LIN[4], Fun-Chi TSAO[2]

[1]*Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City 700000, Vietnam; nguyenhoangchinh@tdt.edu.vn*
[2]*Department of Horticulture and Biotechnology, Chinese Culture University, Taipei 114, Taiwan; rlin@faculty.pccu.edu.tw; hmy6@ulive.pccu.edu.tw (*corresponding author); hsiungan@faculty.pccu.edu.tw; theresapi@yahoo.com.tw*
[3]*Biodiversity Research Center, Academia Sinica, Taipei 115, Taiwan; cmyang@gate.sinica.edu.tw; damnos@gmail.com*
[4]*Graduate Institute of Biotechnology, Chinese Culture University, Taipei 114, Taiwan; lycnthu@gmail.com*
[a,b]*These authors contributed equally to this work*

Abstract

Phalaenopsis (*Phal.*) orchids including white, yellow, and purple flowers are some of the most important commercial orchids worldwide. These flowering plants can be considered to be promising sources of antioxidants since several medicinal orchids were shown to have potential antioxidant activities. The antioxidant activities and several secondary metabolite compounds of the methanolic extracts of four parts (the root, pedicel, leaf, and flower) of three hybrids of white (*Phal.* 'City More'), yellow (*Phal.* 'Sogo Meili'), and purple (*Phal.* 'Queen Beer') flowering orchids were investigated. Results showed that the highest levels of chlorophyll *a* and chlorophyll *b* were respectively obtained in leaf extracts of white and purple orchids, whereas carotenoid showed the highest content in the flower extract of the yellow orchid. Among all tested extracts, flavonoids and anthocyanin demonstrated the highest levels in the flower extract of the purple orchid, whereas the highest level of polyphenols was observed in the flower extract of the yellow orchid. The leaf extract of the white orchid was the most effective extract with a 50% inhibitory concentration in the DPPH-scavenging activity assay, while the highest ferrous iron-chelating effect was observed in flower extracts of the yellow orchid and purple orchid, and the pedicel extract of the purple orchid. In the reducing power assay, the flower extract of the white orchid showed the most potent extract, followed by the leaf extract of the yellow orchid and the flower extract of the purple orchid. Relationships between flower colors and antioxidant activities of these orchids showed them to be potential sources of antioxidants for both medicinal use and stress-tolerance in these orchids.

Keywords: flowering orchid; natural antioxidants; pigments; plant extract; secondary metabolite compound

Introduction

The autoxidation process and oxidative stresses cause various problems in human health, such as ageing, cancer, diabetes, and cardiovascular diseases (Sikora *et al.*, 2008; Huang *et al.*, 2016). To address these problems, many synthetic antioxidants have been used worldwide to prevent the autoxidation process and oxidative stress (Prasad *et al.*, 2009). However, the use of such compounds sometimes has adverse side effects on human health (Prasad *et al.*, 2009; Prasad *et al.*, 2010; Brewer, 2011); thus, efforts have been made to replace these conventional antioxidants. As a result, the use of plant products as natural antioxidants in reducing free radical-induced tissue injury has attracted much attention in recent years (Pokorný, 2007; Brewer, 2011). These natural antioxidants have been widely proven to be potential medicines to maintain health, prevent oxidative stress-mediated diseases, and delay ageing processes (Djeridane *et al.*, 2006; Beta *et al.*, 2017; Caleja *et al.*, 2017). Moreover, these antioxidant resources can be used in cosmetics and foods to enhance defense systems (Chen *et al.*, 2016; Hübner *et al.*, 2016). Therefore, the search for new and safe antioxidants from natural sources is the objective of continued investigations.

Orchids, rated as the most diverse species of all angiosperm families, comprise over 25,000 species (Zahara *et al.*, 2016), among which, terrestrial, epiphytic, and

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saprophytic species are the dominant types of orchids (Rashmi *et al*., 2015). They are distributed worldwide with various diversities in floral parts such as shape, color, and size of the flower (Rashmi *et al*., 2015). These orchids play important economic roles since they are widely cultivated for ornamental purposes (Tsai *et al*., 2015). In addition, several medicinal orchids have been used as food and traditional medicine as anti-inflammatory (Chinsamy *et al*., 2014), anti-cholinesterase (Chinsamy *et al*., 2014), antibacterial (He *et al*., 2016), and anticancer treatments (Sun *et al*., 2016). Recently, various plant parts of medicinal orchids such as *Anacamptis pyrimidalis* (Štajner *et al*., 2010), *Habenaria edgeworthii* (Giri *et al*., 2012), *Ansellia africana* (Chinsamy *et al*., 2014), *Eulophia petersii* (Chinsamy *et al*., 2014), and *Dendrobium nobile* (Bhattacharyya *et al*., 2016), were reported to be potential sources of antioxidants. Various phytochemicals with high biological activity, including carotenoids, flavonoids, phenolics, and their derivatives were found in orchid extracts that showed powerful antioxidant activities (Štajner *et al*., 2010). The antioxidant activities of these compounds are based on scavenging diverse reactive oxygen species (ROS), including peroxyl radicals, hydroxyl radicals, hypochlorous acid, superoxide anions, and peroxynitrite, thus protecting the human body against oxidative damages (Chao *et al*., 2014). Orchids are therefore considered to be promising natural sources of antioxidants (Chinsamy *et al*., 2014).

Phalaenopsis orchids are some of the most important commercial floriculture species in the world because of their beautiful flowers and prolonged blooming (Hsing *et al*., 2016). These orchids have been increasingly marketed worldwide as cut flowers and potted plants and contribute 70% to the value of wholesale flowering plant sales (Guo *et al*., 2012; Zahara *et al*., 2016). Recently, hybrids with improved characteristics have been developed, and interspecific hybridization has been achieved to produce cultivars with improved flower colors (Tatsuzawa *et al*., 2010). Many varieties with various flower colors, including white, orange-red, red, pink, and reddish-purple have been developed. Nevertheless, there is limited information about the relationships between flower color and antioxidant activity of these plants. Of those hybrids, the white (*Phal*. 'City More'), yellow (*Phal*. 'Sogo Meili'), and purple (*Phal*. 'Queen Beer') flowering orchids are the most popular orchids (Tsai *et al.,* 2015) and are mostly imported from Thailand and Taiwan (Guo *et al*., 2012; Zahara *et al*., 2016). Presently, Taiwan is the leading country for exporting *Phalaenopsis* orchids and has produced over 80 million *Phalaenopsis* products from tissue culture (Chen, 2015). However, besides being used for ornamental purposes, these plants can be considered to be promising sources of antioxidants. Although several studies were conducted on medicinal orchids as potential sources of antioxidants (Giri *et al*., 2012; Chinsamy *et al*., 2014; Bhattacharyya *et al*., 2015a), no study has reported the antioxidant capacity among various flower colors of orchid plants. A better understanding of these relationships would aid the effective selection processes of novel flower colors of *Phalaenopsis* breeding programs in the future. Higher levels of antioxidants would also provide protective mechanisms that allow orchid plants to tolerate stressful environments. The aim of our study was to investigate the antioxidant activities of the methanolic extracts from the root, leaf, pedicel, and flower of three Taiwanese flowering orchids (*Phal*. 'City More', *Phal*. 'Sogo Meili', and *Phal*. 'Queen Beer'). Moreover, levels of chlorophyll, anthocyanin, polyphenols, and total flavonoids in the aforementioned tissues of orchids were also examined.

Materials and Methods

Plant materials and preparation of plant extracts

Four parts (the root, pedicel, leaf, and flower) of three hybrids of white (*Phal*. 'City More'), yellow (*Phal*. 'Sogo Meili'), and purple (*Phal*. 'Queen Beer') flowering orchids in 3-inch (7.6 cm) plastic pots were used (Fig. S1). All plants were purchased from a local commercial entity, the Taipei Flower Auction (Neihu District, Taipei City, Taiwan). Pots containing commercial peat moss were placed in a controlled-environment greenhouse under an 8-h photoperiod at 28/22 °C day/night temperatures and a relative humidity of 80%. They were evenly spaced to encourage similar growth rates and sizes. Plants were watered once a week, and an optimal amount of a compound fertilizer solution ($N:P_2O_5:K_2O$, 20:20:20) was applied once every 2 weeks. Plants were maintained for 1 month, and those of a uniform size were then chosen for the antioxidant activity analysis. The experiment was performed twice independently with a randomized design for the growth environment, sampling day, and physiological analyses.

To extract compounds, 0.25 g of dry powder of each plant part was immersed in 5 ml methanol at room temperature. The liquid phase was then separated from the cell debris through filtration under a vacuum using filter paper (Whatman No. 1) to obtain the crude orchid extract which was used for further experiments.

Determination of chlorophyll, carotenoid, anthocyanin, total flavonoid, and polyphenols

Chlorophyll (Chl) and carotenoid (Car) contents were determined using methods described by Porra *et al.* (1989). Briefly, 0.01 g of orchid dry power was mixed with 5 mL of 80% acetone at 4 °C overnight, followed by centrifugation at 13,000 *g* for 5 min to obtain the supernatant. The supernatants were then tested to determine the absorbance of Chl *a* and Chl *b* in acetone at 663.6 and 646.6 nm, respectively, using a Hitachi U-2000 type spectrophotometer (Tokyo, Japan). Concentrations of Chl *a*, Chl *b,* and carotenoid were calculated using the following equations:

Chl $a = (12.25 \times OD_{663.6} - 2.55 \times OD_{646.6}) \times$ volume of supernatant (ml) / sample weight (g);

Chl $b = (20.31 \times OD_{646.6} - 4.91 \times OD_{663.6}) \times$ volume of supernatant (ml) / sample weight (g);

Car = $[(4.69 \times OD_{440.5} \times$ volume of supernatant (ml) / sample weight (g)$] - 0.267 \times$ (Chl *a* + Chl *b*).

Anthocyanin contents of the extracts were measured based on the protocol of Mancinelli *et al.* (1975). A mixture

of 99% methanol containing 1% HCl was added to powder samples and incubated for 1 h at room temperature. The mixture was then centrifuged at 4 °C and 3000 rpm for 5 min to obtain the supernatant. The supernatant was then used to measure absorbances at 530 and 657 nm on a spectrophotometer. The following equation was used:

Anthocyanin content (μmol.g^{-1} DW) = (A_{530} - 0.33 × A_{657} / 31.6) × volume of supernatant (ml) / sample weight (g).

Total flavonoids in the orchid extracts were determined by the method of Djeridane *et al.* (2006). One milliliter of methanol extract (0.5 mg.ml^{-1}) was mixed with 1 ml aluminum chloride (2%). The mixture was stirred and kept at room temperature for 15 min. The absorbance was measured at 430 nm using a spectrophotometer. Quercetin was used as a reference standard, and the total flavonoid content was expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE.g^{-1} DW).

Polyphenol content was determined according to the method of Taga *et al.* (1984). Briefly, standard gallic acid and an aliquot of the acidic methanolic extract were diluted with acidified methanol solution containing 1% HCl. Two ml of 2% Na_2CO_3 were mixed into each sample of 100 μl and allowed to equilibrate for 2 min before adding 50% Folin-Ciocalteu reagent. Absorbance at 750 nm was measured at room temperature using the Varioskan Flash Multimode Reader (Thermo Scientific, Rockford, IL, USA). The standard curve for gallic acid was used to calculate polyphenol levels. Total phenolics were expressed as the mg gallic acid equivalent (GAE).g^{-1} of dry weight. The standard curve equation was $y = 0.4995x - 0.011$, where $R^2 = 0.9944$.

Determination of the Antioxidant Activity

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

DPPH free radical-scavenging activities of the orchid extracts were determined according to the method of Yoshiki *et al.* (2001) with slight modifications. Aliquots of serial dilutions of 0.05 ml of the methanol extract were added to 0.15 ml of a DPPH solution (0.4 mM in methanol). The mixture was then well mixed and left at room temperature for 90 min in the dark before measuring the absorbance at 517 nm using a Hitachi U-2000 type spectrophotometer. The radical-scavenging activity was calculated using the following equation:

Scavenging activity (%) = [1 - (OD_1 sample - OD_2 sample) / (OD_3 control - OD_4 control)] × 100%.

Methanol was used instead of a sample as the control. The OD_1 sample contained added DPPH in the experimental group, but the OD_2 sample did not contain added DPPH in the experimental group. The OD_3 control contained added DPPH in the control (methanol), whereas the OD_4 control did not contain added DPPH in the control (methanol). A calibration curve was constructed using different concentrations (10~100 μM) of butylated hydroxytoluene (BHT) as a positive control. The concentration required for a 50% decrease in the absorbance of DPPH radicals (IC_{50}) was then calculated as the percent inhibition of DPPH by plotting the percentage of residual DPPH at a steady state as a function of the sample extract concentration.

Determination of the ferrous iron-chelating ability

The ferrous iron-chelating ability was determined based on the method of Dinis *et al.* (1994). Briefly, an aliquot of 1 ml of the methanol extract was added to 3.7 ml of 80% methanol containing 100 μl of 2 mM $FeCl_2{\cdot}4H_2O$. The mixture was allowed to sit for 30 s, followed by the addition of 200 μl of a 5 mM ferrozine solution, and then incubated for 10 min at room temperature before the absorbance was measured at 562 nm. The ferrous iron-chelating ability was calculated as follows:

Ferrous iron-chelating ability (%) = [1 - (OD_1 sample - OD_2 sample) / (OD_3 control - OD_4 control)] × 100%.

Methanol was used instead of a sample as the control. The OD_1 sample contained added DPPH in the experimental group, but the OD_2 sample did not contain added DPPH in the experimental group. The OD_3 control contained added DPPH in the control (methanol), whereas the OD_4 control did not contain added DPPH in the control (methanol). A standard curve was made using 10~100 μM ethylenediaminetetraacetic acid (EDTA) as a positive control. The concentration required for a 50% decrease in the absorbance of ferrous iron (IC_{50}) was calculated as the percent inhibition of ferrous iron by plotting the percentage of residual ferrous iron in a steady state as a function of the sample extract concentration.

Measurement of the reducing power

The reducing power of the extracts was determined as described by Pulido *et al.* (2000). A sample extract (100 mg) in 2 ml methanol was mixed with phosphate buffer (50 μl, 0.2 M, pH 6.6) and potassium ferricyanide (50 μl, 1%) and the mixture was incubated at 50 °C for 20 min. Trichloroacetic acid (50 μl, 10%) was added to the reaction mixture, which was then centrifuged at 3000 rpm for 10 min. The supernatant (90.9 μl) was mixed with an equal volume of distilled-deionized water (d.d.H_2O) and 18.2 μl of 0.1% ferric chloride. After 10 min, the absorbance at 700 nm was measured against a blank. The reduction capacity was calculated as follows:

Reduction capacity (%) = [(OD sample - OD control) / OD standard] × 100%.

Methanol was used instead of a sample as the control, and BHT in methanol was used as the standard. The concentration required for a 50% decrease in the absorbance of the reducing power (IC_{50}) was then calculated as the percent inhibition of the reducing power by plotting the percentage of the residual reducing power in a steady state as a function of the sample extract concentration.

Statistical analysis

Data were determined in triplicate, and results are expressed as the mean ± standard deviation (SD). An analysis of variance (ANOVA) with the least significant difference (LSD) test at $p \leq 0.05$ was performed using SAS vers. 9 (SAS Institute, Cary, NC, USA).

Results

Photosynthesis pigments and secondary metabolite compounds among orchid extracts

Table 1 presents the contents of Chl *a*, Chl *b*, Chl *a*+*b*,

Chl *a/b*, carotenoids, flavonoids, polypenols, and anthocyanin in extracts of different parts of the orchids. Results show that in all orchid species tested, levels of Chl *a*, Chl *b*, total Chl, and carotenoids in leaves were significantly higher than those of other plant parts in each plant, while the flowers contained a higher ratio of Chl *a/b* compared to other parts due to the Chl *b* lower contents. Of those orchid extracts tested, the highest contents of Chl *a* (2.19 ± 0.42 mg.g^{-1} DW) and Chl *b* (1.06 ± 0.31 mg.g^{-1} DW) were obtained in leaf extracts of white and purple orchids, respectively, whereas carotenoid reached the highest level (0.82 ± 0.02 mg.g^{-1} DW) in the methanolic flower extract of the yellow orchid. In addition, among all examined samples, the highest flavonoid and anthocyanin contents were found in the flower extract of the purple orchid at 138.70 ± 3.03 mg QE.g^{-1} DW and 147.48 ± 11.85 µg.g^{-1} DW, respectively. However, the flower extract of the white orchid had low flavonoid (8.10 ± 1.06 mg QE.g^{-1} DW) and anthocyanin (12.01 ± 4.71 µmol.g^{-1} DW) contents compared to other parts. Leaf extracts of the yellow orchid contained significantly higher flavonoid (51.59 ± 8.05 mg QE.g^{-1} DW) and anthocyanin (40.57 ± 3.73 µmol.g^{-1} DW) contents than the other parts. Flower (446.22 ± 60.03 mg gallic acid.g^{-1} DW) and leaf (244.23 ± 51.39 mg gallic acid.g^{-1} DW) of yellow plant contained significantly higher levels of polyphenols compared to other plants.

Antioxidant activities among the orchid extracts

The antioxidant activities of orchid extracts were measured as DPPH radical-scavenging activity, the ferrous iron-chelating ability, and a ferric-reducing power assay. As shown in Fig. 1, different parts of the orchid plants possessed different free radical-scavenging activities. Leaf extracts of white and yellow orchids had significant higher DPPH radical scavenging activities (5~25 mg.ml^{-1}) than other parts in each species. However, in the purple orchid, significantly lower radical-scavenging activity (1~50 mg.ml^{-1}) was observed in the pedicel extract. Furthermore, among the three species tested, the leaf extract of the white orchid was the most potent extract with an IC_{50} of 8.01 ± 0.97 mg.ml^{-1}, followed by the yellow orchid (IC_{50} = 12.73 ± 0.37 mg.ml^{-1}) (Table 2). Compared to white and yellow orchids, all parts of the purple orchid demonstrated a lower ability to scavenge DPPH with IC_{50} values of 16.91 ± 3.54~32.63± 1.84 mg.ml^{-1}.

Fig. 2 shows that the ferrous iron-chelating effect significantly differed among orchid extracts. The flower extracts of white and yellow species displayed significantly higher iron-chelating rates (5~10 mg.ml^{-1}) than other parts. Moreover, both the flower and pedicel extracts of the purple species exhibited significantly higher iron-chelating rates (1~10 mg.ml^{-1}) than those of the leaves and roots. The highest ferrous iron-chelating effect was observed in flower extracts of the yellow orchid (IC_{50} =1.02 ± 0.27 mg.ml^{-1}) and purple orchid (IC_{50} = 1.50 ± 0.23 mg.ml^{-1}), and the pedicel extract of the purple orchid (IC_{50} of 1.71 ± 0.33 mg.ml^{-1}) (Table 2).

Fig. 3 demonstrates various levels of reducing powers in different plant parts. Both the flower and leaf extracts of white and yellow species exhibited higher reducing power activities (5~50 mg.ml^{-1}) than the root and pedicel in each plant, whereas the flower extract of the purple orchid showed higher reducing power activity (5~50 mg.ml^{-1}) than the other parts. Of all samples tested, the flower extract of the white orchid was the most effective in reducing activity with an IC_{50} of 15.02 ± 1.12 mg.ml^{-1}, followed by the leaf extract of the yellow orchid (IC_{50} = 20.46 ± 0.98 mg.ml^{-1}) and the flower extract of the purple orchid (IC_{50} = 23.53± 1.02 mg.ml^{-1}).

Table 1. Content of pigments, flavonoids, polyphenol, and anthocyanin in extracts of different parts of orchids

Species	Part	Chl *a* (mg.g^{-1} DW)	Chl *b* (mg.g^{-1} DW)	Chl *a+b* (mg.g^{-1} DW)	Chl *a/b*	Car (mg.g^{-1} DW)	Fla (mg QE.g^{-1} DW)	Pph (mg GAE.g^{-1} DW)	Ant (µmol.g^{-1} DW)
Phal. 'City More'	Root	0.56 ± 0.08c	0.27 ± 0.05c	0.83 ± 0.13d	2.07 ± 0.11bc	0.32 ± 0.06e	17.54 ± 4.00ef	115.96 ± 13.17de	13.19 ± 2.81ef
	Pedicle	0.51 ± 0.09c	0.23 ± 0.04c	0.74 ± 0.13d	2.29 ± 0.11b	0.23 ± 0.05f	29.17 ± 3.43c	89.85 ± 8.43e	30.05 ± 5.07c
	Leaf	2.19 ± 0.42a	0.96 ± 0.21a	3.15 ± 0.63a	2.28 ± 0.06b	0.79 ± 0.10ab	49.12 ± 6.35b	92.75 ± 9.54e	32.60 ± 6.32c
	Flower	0.07 ± 0.02e	0.02 ± 0.01d	0.09 ± 0.03f	3.14 ± 0.87a	0.14 ± 0.23g	8.10 ± 1.06g	110.48 ± 16.68de	12.01 ± 4.71ef
Phal. 'Sogo Meili'	Root	0.42 ± 0.10cd	0.23 ± 0.05c	0.64 ± 0.15de	1.84 ± 0.08c	0.39 ± 0.07de	32.91 ± 7.57c	118.48 ± 31.42de	23.16 ± 5.39d
	Pedicle	0.53 ± 0.07c	0.27 ± 0.04c	0.80 ± 0.11d	2.01 ± 0.07bc	0.24 ± 0.03f	12.82 ± 0.29f	153.43 ± 30.91cd	16.71 ± 2.52ef
	Leaf	1.80 ± 0.08b	0.91 ± 0.05a	2.71 ± 0.13b	1.99 ± 0.04bc	0.78 ± 0.02ab	51.59 ± 8.05b	244.23 ± 51.39b	40.57 ± 3.73b
	Flower	0.25 ± 0.01de	0.12 ± 0.03cd	0.37 ± 0.04ef	2.23 ± 0.34b	0.82 ± 0.02a	21.62 ± 3.58de	446.22 ± 60.03a	14.45 ± 3.07ef
Phal. 'Queen Beer'	Root	0.15 ± 0.03e	0.44 ± 0.09b	0.59 ± 0.12de	0.34 ± 0.02e	0.5 ± 0.06c	15.40 ± 2.37ef	115.00 ± 22.92de	7.26 ± 0.68g
	Pedicle	0.15 ± 0.05e	0.48 ± 0.16b	0.63 ± 0.20de	0.30 ± 0.01e	0.38 ± 0.04e	25.88 ± 2.52cd	78.24 ± 11.28e	16.61 ± 1.26ef
	Leaf	0.26 ± 0.05d	1.06 ± 0.31a	1.31 ± 0.35 c	0.25 ± 0.02e	0.46 ± 0.02cd	32.02 ± 6.41c	124.67 ± 16.41de	19.83 ± 1.77de
	Flower	0.05 ± 0.01e	0.11 ± 0.02cd	0.16 ± 0.03f	0.52 ± 0.11d	0.22± 0.04f	138.70 ± 3.03a	179.61 ± 43.75c	147.48 ± 11.85a

All values are the mean ± SD (n = 3~5). Means within a column with different letters significantly differ by Duncan's test at p <0.05.

Table 2. The 50% inhibitory concentration (IC50) values of DPPH radical-scavenging activities, ferrous iron-chelating abilities, and ferric-reducing power assay of extracts of different parts of orchids

Species	Part	DPPH scavenge (mg.ml^{-1})	Fe^{2+}-chelating effect (mg.ml^{-1})	Reducing power rate (mg.ml^{-1})
Phal. 'City More'	Root	18.20 ± 0.78cd	6.60 ± 0.79cd	34.29 ± 3.60bc
	Pedicel	15.13 ± 1.57de	35.88 ± 5.58a	44.04 ± 8.45a
	Leaf	8.01 ± 0.97g	3.44 ± 0.38d	27.42 ± 2.34c
	Flower	15.51 ± 1.22de	3.25 ± 0.22d	15.02 ± 1.12f

Phal. 'Sogo Meili'	Root	17.86 ± 0.83d	7.51 ± 0.47c	36.25 ± 1.24b
	Pedicel	30.42 ± 3.66a	3.40 ± 0.58d	44.48 ± 8.10a
	Leaf	12.73 ± 0.37f	24.40 ± 5.56b	20.46 ± 0.98e
	Flower	14.79 ± 0.38e	1.02 ± 0.27e	33.66 ± 5.64bc
Phal. 'Queen Beer'	Root	19.66 ± 0.73cd	5.81 ± 0.25cd	33.74 ± 4.13bc
	Pedicel	32.63 ± 1.84a	1.71 ± 0.33e	37.31 ± 6.69b
	Leaf	21.24 ± 1.52b	5.73 ± 0.86cd	31.32 ± 1.73bc
	Flower	16.91 ± 3.54de	1.50 ± 0.23e	23.53 ± 1.02d
Standard ($\mu g.ml^{-1}$)		0.51± 0.08	2.16± 0.16	3.76± 0.18

All values are the mean ± SD (n = 3~5). Means within a column with different letters significantly differ by Duncan's test at $p < 0.05$.

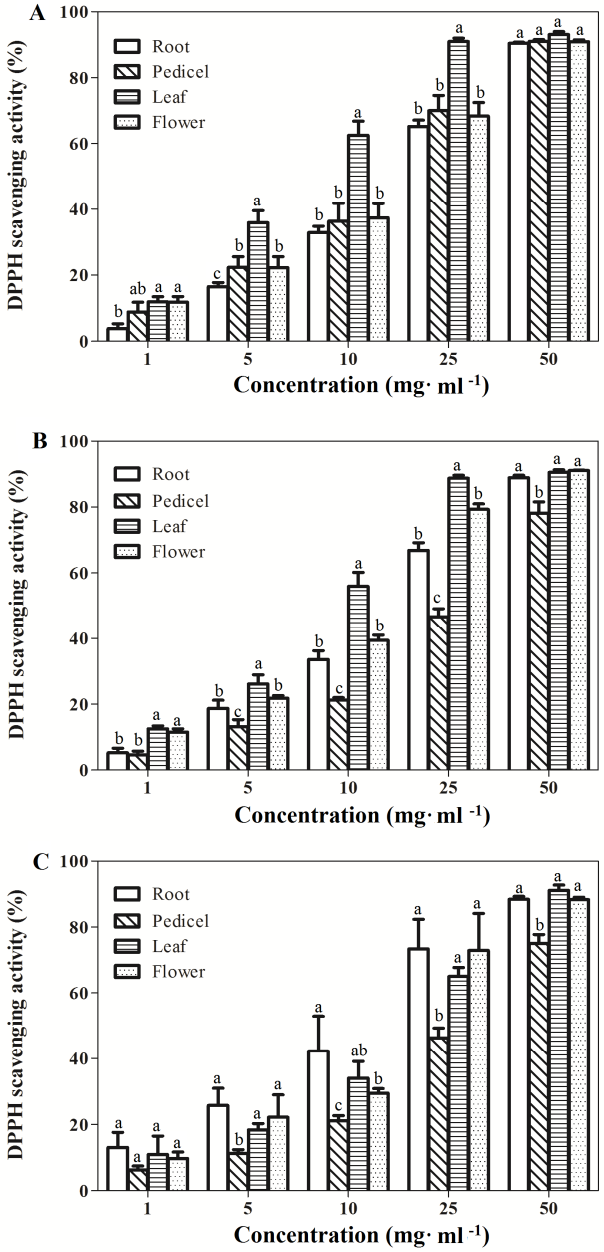

Fig. 1. DPPH-scavenging activities of white (*Phal.* 'City More', Panel A), yellow (*Phal.* 'Sogo Meili', Panel B), and purple (*Phal.* 'Queen Beer', Panel C) orchid extracts. Vertical bars indicate the standard deviation (n = 5). a, b, and c: different characters represent significant difference by Duncan's test at p < 0.05

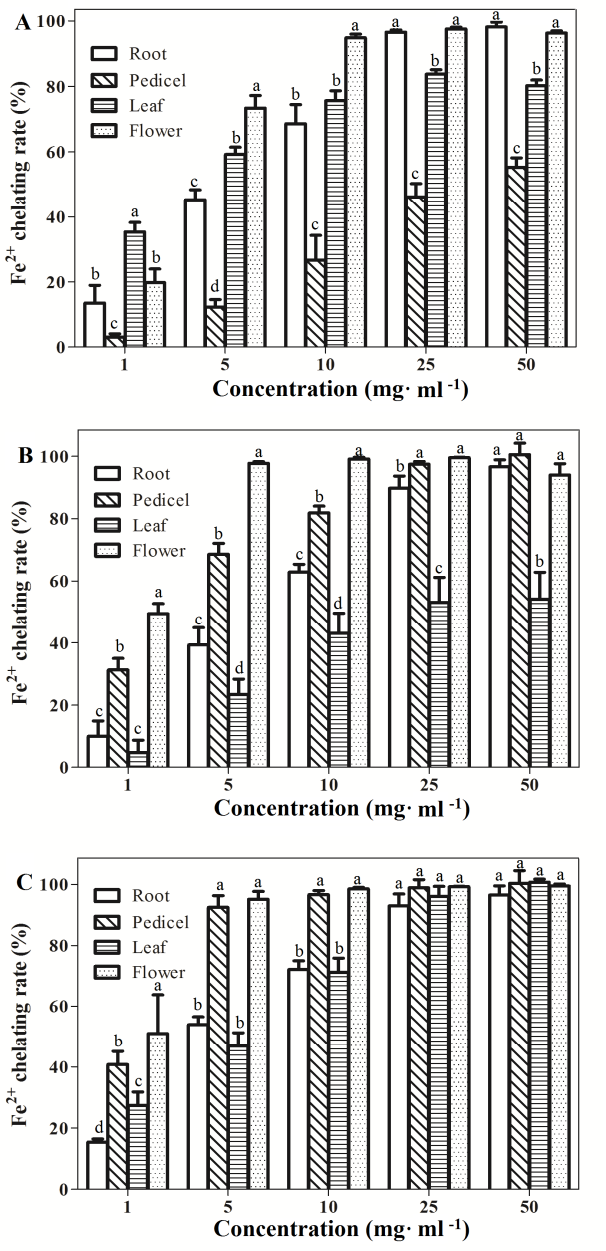

Fig. 2. Fe^{2+}-chelating abilities of white (*Phal.* 'City More', Panel A), yellow (*Phal.* 'Sogo Meili', Panel B), and purple (*Phal.* 'Queen Beer', Panel C) orchid extracts. Vertical bars indicate the standard deviation (n = 5). a, b, c and d: different characters represent significant difference by Duncan's test at p < 0.05

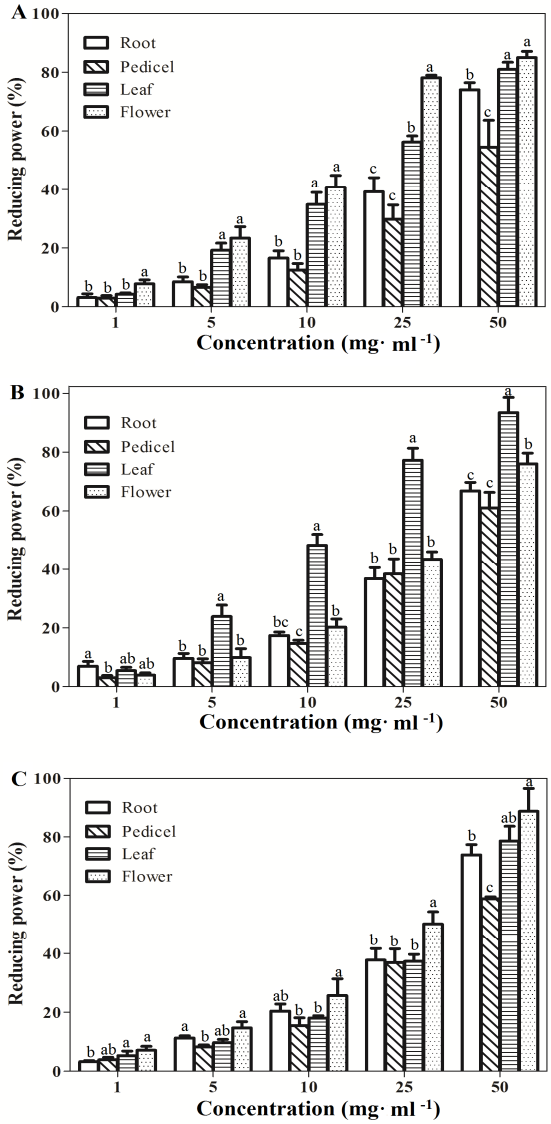

Fig. 3. Reducing power activities of white (*Phal.* 'City More', Panel A), yellow (*Phal.* 'Sogo Meili', Panel B), and purple (*Phal.* 'Queen Beer', Panel C) orchid extracts. Vertical bars indicate the standard deviation (n = 5). a, b, and c: different characters represent significant difference by Duncan's test at $p < 0.05$

Discussion

The Orchidaceae is one of the largest families of flowering plants, and many of its members have been developed worldwide for market use. Several medicinal orchid species were found to contain many phytochemicals, such as phenols, terpenoids, flavonoids, alkaloids, and steroids in either plant parts or the entire plants (Štajner *et al.*, 2010; Bhattacharyya *et al.*, 2015b). In our study, different plant parts of three Taiwanese orchid hybrids displayed wide variations in photosynthetic pigments, flavonoids, polyphenols, and anthocyanin contents as well as antioxidant activities. In general, leaves of all tested orchids were rich in Chl *a* and *b*, and carotenoids, indicating that light is necessary for the synthesis of pigments. White orchid leaves contained more Chl *a*, Chl *b*, carotenoids, flavonoids, and anthocyanin levels than other tested parts. Yellow orchid leaves also had similar trends except for carotenoid. Both flower and leaf of yellow orchid were abundant in polyphenols Purple orchid flowers contained higher flavonoids and anthocyanin compared to other parts. On the other hand, all flowers showed a higher Chl *a/b* ratio than other parts, because significantly low Chl *b* contents were observed in these flowers. Differences in pigment contents of plant parts might be due to light absorption affecting pigment synthesis, thus causing lower Chl *a* and *b* in roots, flowers, and pedicels compared to leaves. High contents of pigments in leaves of these orchid hybrids could be attributed to the wide existence of those hybrids even under high light and radiation conditions of the environment, since those compounds were reported to play important roles against stressful conditions (de la Rosa-Manzano *et al.*, 2015). A better understanding of the relationships of flower colors with pigment, flavonoid, polyphenols, and anthocyanin contents will stimulate more-efficient breeding of these genera. Further analyses of more pigments and secondary metabolite compounds in other orchid genera may contribute to progress in chemotaxonomic and phylogenic studies in the Orchidaceae.

There has been increasing interest in using antioxidants from plants as functional foods and nutraceutical products with antioxidant properties (Brewer, 2011). Various species

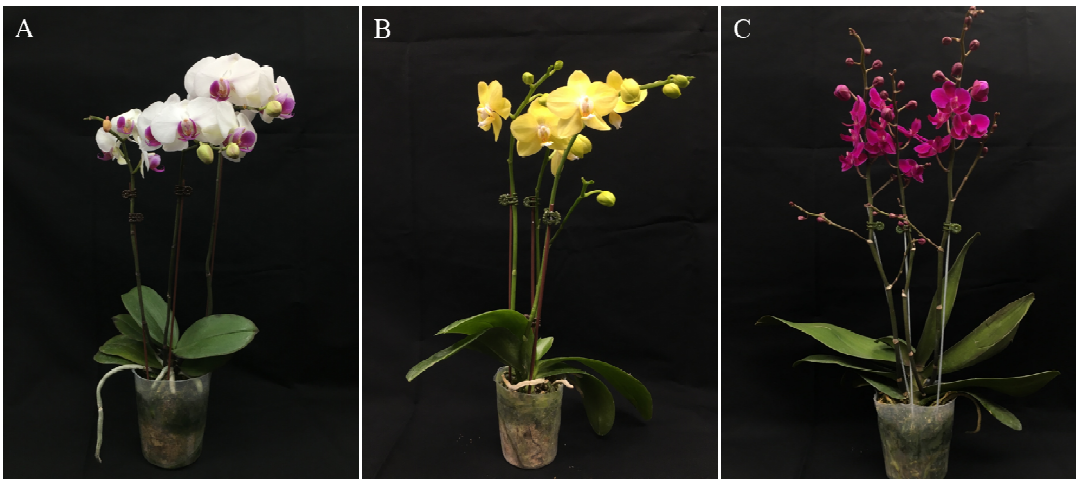

Fig. S1. White (*Phal.* 'City More', Panel A), yellow (*Phal.* 'Sogo Meili', Panel B), and purple (*Phal.* 'Queen Beer', Panel C) orchids were used as plant materials

of orchids were recently reported to be sources of antioxidants (Chinsamy *et al.*, 2014; Schuster *et al.*, 2017). In this study, the antioxidant activities of different plant parts of three orchid species were studied using DPPH-scavenging activity assays since free radical scavenging is one of the mechanisms by which antioxidants inhibit lipid oxidation and prevent oxidative damage (Huang *et al.*, 2017). Flower extracts of the white and yellow species provided significantly higher iron-chelating rates to prevent the formation of ferrozine from ferrous ions than other parts, indicating that those flower extracts were excellent chelators of ferrous ions. The white orchid leaf extract showed the highest IC_{50} value of DPPH-scavenging activity, while flower extracts of the yellow and purple orchids were the best chelators of ferrous ions in terms of IC_{50} values of the ferrous iron-chelating ability assay. Alternatively, the flower extract of the white orchid was most potent in terms of the IC_{50} of the reducing power assay.

Among all samples tested, flower and leaf extracts possessed higher antioxidant activities than pedicel and root extracts within each species. These flowers and leaves contained higher levels of flavonoids and anthocyanin than those of roots and pedicels, since flavonoids and anthocyanin mainly contribute to antioxidant activities (Chao *et al.*, 2014; Bhattacharyya *et al.*, 2016). The presence of the highest flavonoids and anthocyanin in the flower extract of the purple orchid could have played a role in the antioxidant activity observed in the ferrous iron-chelating ability assay. Unlike the DPPH-scavenging activity, the purple orchid was observed to exhibit higher antioxidant activity in the ferrous iron-chelating assay than did the yellow and white orchids, indicating that different extracts have different antioxidant mechanisms. The pedicel and root extracts of all species exhibited lower reducing power activities than extracts from other parts in each plant, indicating that the pedicles and roots have low efficiency in antioxidant activities compared to other plant parts. This might be due to low light absorption in roots and pedicels. The light that plants receive often exceeds the amount needed for photosynthesis, and excess light may cause foliar damage because it increases the probability of the formation of highly oxidizing radicals as ROS (Ledford and Niyogi, 2005). To counteract the formation of these compounds, plant tissues possess different photoprotective mechanisms to remove excess energy or to avoid excess heat, which include leaf and chloroplast movements, carotenoid production, photosystem II heat dissipation of absorbed energy, and heat flux by conduction and convection (Takahashi and Badger, 2011), which reduce the probability of damage to the photosynthetic apparatus. The photoprotective mechanisms may allow orchids to survive in these environments. It is worth investigating how root and foliar concentrations of antioxidants change in different orchid species in response to light intensity changes, and develop different strategies to cope with common environmental challenges for orchids.

The use of orchids as traditional medicines for treating various diseases, such as stomach ache, body ache, headache, fever, and cardiac and nervous disorders was found in Asia, the Americas, Africa, and Europe (Bulpitt, 2005; Pant, 2013). Although many studies on bioactive compounds and pharmaceutical properties of orchids have been conducted, most of that work focused on leaves and stems of medicinal orchid species. This study investigated the pigments and several phytochemicals in various parts of orchids as well as their activities. Our results demonstrated that the flavonoid and anthocyanin contents in tested orchids are much higher than those of many Taiwanese vegetables, including sweet potato (*Ipomoea batatas* L.), *Abelmoschus esculentus* Moench, *Anisogonium esculentum*, *Toona sinensis*, *Asplenium antiquum* Makino, *Saccharum officinarum*, *Hemerocallis fulva*-green, *Nymphoides cristata*, *Sechium edule*, *Sechium edule*, and *Momordica charantia* (Huang *et al.*, 2006; Chao *et al.*, 2014). In addition, the antioxidant activity of tested orchids is also comparable to that of sweet potato (Huang *et al.*, 2006; Rumbaoa *et al.*, 2009). The highest total flavonoid values reported in the USDA database (2010) for raw vegetables are capers (*Capparis*, 28.99 $mg.g^{-1}$ DW), parsley (11.85 $mg.g^{-1}$ DW), lovage (*Levisticum officinale*, 17.7 $mg.g^{-1}$ DW), dill weed (*Anethum graveolens*, 6.12 $mg.g^{-1}$ DW), and dock (*Rumex* spp., 5.10 $mg.g^{-1}$ DW). From the findings of this study, orchid plants after production of commercial flowers are suggested to be used for health food and medicinal purposes due to their bioactive compounds and antioxidant activities.

Conclusions

Orchids have been grown worldwide and play important economic roles. Three flowering orchids, *Phal.* 'City More', *Phal.* 'Sogo Meili', and *Phal.* 'Queen Beer', showed potential antioxidant activities due to their high contents of carotenoids, flavonoids, and anthocyanin. The results provide a new direction in the use of flowering orchids as low-cost antioxidant sources, and orchids could be promising materials for the pharmaceutical and neutraceutical industries.

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