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Influence of Storage Temperature and Duration on Phytochemical Contents and Antioxidant Activities of the Inflorescence of *Aranda* Hybrids

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ABSTRACT

Background: Four *Aranda* hybrids, including *Aranda* Bangkhuntian Gold, *A.* Calypso, *A.* Royal Sapphire and *A.* Sayan Duangporn were analyzed for their bioactive substances and antioxidative activities as commercial cut flowers with suggested medicinal properties.

Methods: A completely randomized design was conducted for this study. Total flavonoids, total phenolics and 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferrous ion (Fe (II)) radical scavenging activities were analyzed from the ethanoic extracts of four parts, including flower bud, blooming flowers, leaves and roots of the four *Aranda* cultivars. Furthermore, the dried powder samples were kept at room temperature (25-28°C) or cool (5-7°C) for six months to compare the bioactive substances and antioxidative activities after storage.

Result: In most *Aranda* hybrids, the leaf ethanolic extracts had the highest total flavonoids and total phenolic contents range from 26.63-40.05 mg rutin equivalent (RUE)/g extract and 152.00-254.13 mg gallic acid equivalent (GAE)/g extract, respectively, which is remarkably different from the floral parts (bud or blooming flowers) that had only 8.00-20.80 mg RUE/g extract and 97.44-143.44 mg GAE/g extract, respectively. The highest ABTS and Fe (II) antioxidant activities were detected in the leaf part with 53.13-78.85% and 11.19-15.07%, respectively, while the inhibitory effect on DPPH of most *Aranda* hybrids was the highest in the root part (67.37-80.90%), which 1.13-2.78 folds higher than the floral parts. Compared among the four *Aranda* hybrids, the *A.* Bangkhuntian Gold had the highest leaf total phenolic content (291.67 mg GAE/g extract) and inhibitory activities on ABTS and DPPH in the root part, about 80.90-82.23%. At six months of dried sample storage at room temperature or in a refrigerator, the total phenolic contents, ABTS, DPPH and Fe (II) activities in all organs of most *Aranda* hybrids could maintain similar levels in both storage temperatures. In contrast, the total flavonoid contents of leaves and roots were more sensitive to room temperature since the level was markedly reduced.

Key words: ABTS, DPPH, Flavonoid, Orchid, Phenolic.

INTRODUCTION

Utilization of natural products from plants for health, medical and beauty-related industries is trendy and increasingly in demand. Orchids are reported to be rich in antioxidant properties. They can be used for medicinal purposes such as antioxidant, antirheumatic, anti-inflammatory, anti-carcinogenic, hypoglycemic, antimicrobial, anticonvulsive, anti-tyrosinase activity, relaxation, neuroprotective, antivirus, *etc*. (Gutiérrez, 2010, Rungsang *et al*., 2023).

Aranda hybrids (formerly called 'Mokara') are monopodial orchids. They are progenies from crossing three orchid genera, including *Arachnis*, *Ascocentrum* and *Vanda*. They have long, parallel-veined, leathery leaves arranged on two opposite sides along the stem from the base to the top of the plant. As a famous cut flower, the inflorescence of *Aranda* hybrids is about 30-50 cm long, composed of 9-19 flowers. Each flower is about 5-8 cm wide and has a long vase life of about 5.8-22 days, depending on the cultivar (Dymek *et al*., 2019). Aerial roots are produced from the axillary buds of internodes. As a climbing orchid, the plant can grow upwards and reach a height of 1.5-2.0 meters when fully mature. The matured plant has a long stem with numerous leaves and aerial roots. Growers need to top-cut the mature *Aranda*'s stem to prevent it from growing too tall, which can make it difficult to harvest the inflorescence. The removed leaves and aerial roots can be used for extracting bio-substances for medicinal purposes without harming the mother plants. Some biological screenings exist

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on stems, roots and leaves of monopodial orchids such as *Vanda coerulea* and some *Phalaenopsis* hybrids. The crude hydro-alcoholic stem extract of *V. coerulea* showed high DPPH/•OH radical scavenging activities that can reduce skin oxidative stress from UV exposure. The extract can inhibit cyclo-oxygenase 2 (COX-2) activity, an enzyme for generating prostaglandin metabolites and cause skin inflammation (Simmler *et al*., 2010). In *Phalaenopsis* hybrids, the ethanol extracted from roots displays higher DPPH/•OH radical scavenging activities than the leaf part. However, the highest total phenolics and total flavonoids were detected from the leaf part. The roots of *Phalaenopsis* can be harvested from the decorative finishing pots to reduce waste. They serve as a potential source of natural antioxidants (Minh *et al*., 2016). Although the *Aranda* is a well-known commercial orchid, the information about the antioxidant properties of these orchids is limited. Accordingly, this study reports the *Aranda* hybrids' total flavonoid, phenolic contents and antioxidant activities. Furthermore, the influence of storage temperature on the antioxidant properties of the dried samples of the *Aranda* hybrids under long storage for six months was investigated. We hypothesize that the root part of the *Aranda* hybrids likely contains the highest level of bioactive substances and antioxidative activities, as reported in some monopodial orchids like the *Phalanopsis* (Minh *et al*., 2016). Additionally, storing the samples in cool conditions for six months may help maintain better antioxidant properties compared to storing them at the room temperature. The finding will provide valuable information for the medicinal utilization of these orchids to add value to the raw materials.

MATERIALS AND METHODS

Plant materials and extraction procedure

Five-year-old *Aranda* hybrids were used in this study consisting of *Aranda* Bangkhuntian Gold, *A*. Calypso, *A*. Royal Sapphire and *A*. Sayan Duangporn (Fig 1). The orchids were grown at a commercial farm in Kratumban district, Samut Sakhon province, Thailand. The orchid parts, including the leaves, roots, floral buds and blooming flowers, were collected, cleaned with distilled water and dried at 70°C for 48 hours in a hot air oven. Dried sample powders were separated to keep at room temperature (25-28°C) and cool temperature (5-7°C) by following experimental treatments. For the preparation of the extracts, one gram of dried sample powder was macerated with 10 mL of ethanol (80%) and incubated at room temperature (25°C). After 24 h, the extracts were filtrated with Whatman® paper filter no. 1. The extracts were diluted with 80% ethanol to be a final concentration of 1000 µg/mL for determining phytochemical contents (total flavonoids and total phenolics) and antioxidant properties (ABTS, DPPH and Fe (II) scavenging activities).

Determination of total flavonoid contents

Total flavonoid content was determined according to the colorimetric assay modified by Djeridane *et al*. (2006). The 100 µL of *Aranda* extracts at a concentration of 1000 µg/mL was mixed with 100 µL of aluminum chloride (2% w/v) in a 96-well plate and incubated at ambient temperature for 15 min. Then, the solution was measured at an absorbance of 430 nm using a microplate reader (Multiskan Go, Thermo Scientific, MA, USA). The total flavonoid content in each

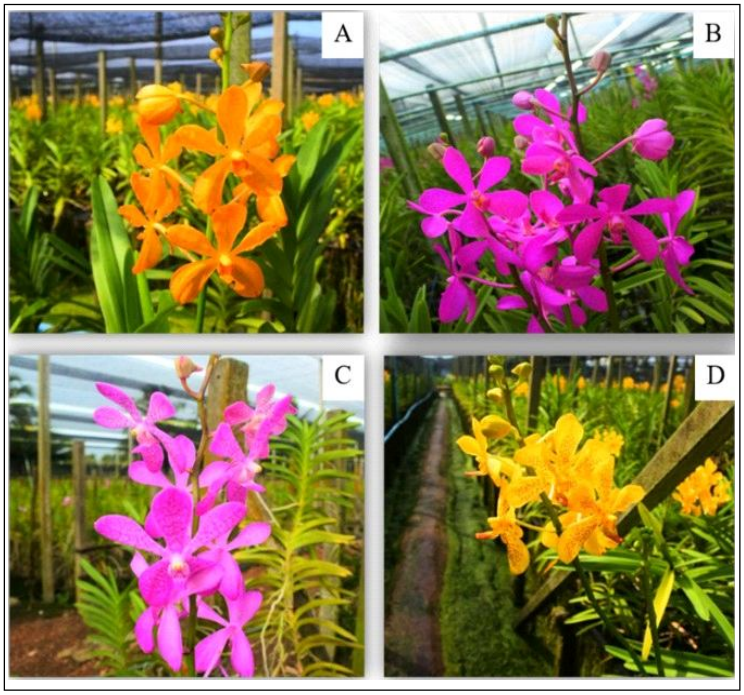


Fig 1: Varieties of plant species *i.e. Aranda* Bangkhuntian Gold (A) *A*. Calypso; (B) *A*. Royal Sapphire; (C) *A*. Sayan Duangporn (D).

extract was compared with the rutin standard curve when Y= 0.007x - 0.003 (R^2= 0.999). The result was expressed as mg of rutin equivalents (RUE) per g of extract (mg RUE/g extract).

Determination of total phenolic contents

The modification method from Siddhuraju and Becker (2003) determined the total phenolic content. The 20 µL of *Aranda* extracts were mixed with 50 µL of distilled water and 50 µL of Folins-phenol reagent (ratio of reagent: water 1:1) in a 96-well plate, then added with 80 µL of sodium carbonate (7.5% w/v). The solution was incubated at ambient temperature for 30 min and measured at an absorbance of 725 nm using a microplate reader. Gallic acid (0.001 to 0.01 mg/mL) was used as a standard. The total phenolic content in each extract was compared with the standard curve when Y=0.0079x + 0.055 (R^2=0.997). The result was expressed as mg of gallic acid equivalents (GAE) per 1 mg of solid crude extract (mg GAE/g extract).

Determination ABTS scavenging activity

The total antioxidant activity of the ethanol extracts was determined by ABTS assay (Hsu *et al*., 2011) with a slight modification. A mixture of 2,2′-azinobis (3-ethylbenzothiazoline -6-sulfonic acid (ABTS) and potassium persulfate were incubated in the dark at room temperature (25°C±2) for induced oxidation to be $ABTS^{\cdot+}$ for 16 hr. It measured the mixture at an absorbance of 734 nm until the absorbance value reached 0.700±0.050. Added the ethanolic *Aranda* extract to the mixture, kept in the dark for 6 min and measured at an absorbance of 734 nm. The tert-butylhydroxytoluene (BHT) (Sigma-Aldrich, Inc., USA) and tocopherol were used as positive control for comparing antioxidant capacity.

Determination DPPH scavenging activity

The total antioxidant activity of the ethanol extracts was determined by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay with a slight modification (Boskou *et al*., 2006). A hundred micro milliliters of *Aranda* extract were mixed with 40 µL of DPPH solution (Sigma-Aldrich, Inc., USA) at 0.5 mM and 100 µL of sodium acetate buffer (0.1 M, pH 5.5). Then, the solution was kept in the dark at room temperature (25°C±2) for 30 min and measured at an absorbance of 517 nm. The tert-butylhydroxytoluene (BHT) (Sigma-Aldrich, Inc., USA) was used as a positive control for comparing antioxidant capacity.

$$\text{Radical scavenging (\%)} = \frac{O.D._{control} - O.D._{sample}}{O.D._{control}} \times 100$$

Where:

O.D.= Optical density in the presence or absence of the samples.

Determination Fe (II) scavenging activity

The reducing ability of the ethanolic extracts was determined following the methods of Oyaizu (1986) with slight modification. The 400 µL ethanolic *Aranda* extract was added to 800 µL of 1% potassium ferricyanide. Then, the solution was incubated at 50°C for 20 min, added with 800 µL of 1% trichloroacetic (TCA) and centrifuged at 5,000 rpm for 10 min. It took only 400 µL of supernatant to mix with 400 µL distilled water and 80 µL of 1% ferric chloride. The solution was measured at an absorbance of 700 nm using the tocopherol as a positive control for comparing antioxidant capacity.

Statistical analysis

Statistical analysis was performed using one-way ANOVA. The data were presented as mean±standard deviation. Mean comparisons were analyzed using Duncan's multiple range test (DMRT) at $p<0.05$. All analyses were performed in four biological replicates.

RESULTS AND DISCUSSION

Phytochemical contents and antioxidant activities of *Aranda* after harvested

After harvesting and separating *Aranda*'s leaves, roots, flower buds and blooming flowers, the samples were dried and promptly analyzed for biological substances and antioxidant activities. The results showed that the total flavonoid content was markedly detected in the leaf part of all *Aranda* hybrids, ranging from 26.63-40.05 mg RUE/g extract, which was 1.48-1.96 folds higher than the roots and the other floral parts. Similarly, the total phenolic content was greatly accumulated in the leaves part, ranging from 152.00-254.13 mg GAE/g extract, 1.23-2.50 folds higher than the floral parts (Table 1). The highest total flavonoid content in leaves has also been reported in aqueous extract of *Thapsia garganica* L. (Djahida and Houcine, 2021) and the ethanolic extract of *Dendrobium sulcatum,* in which both the stem and leaf extracts displayed a higher level of total flavonoids than the floral organs (Rungsan *et al*., 2023). In response to environmental stress, some mature orchids accumulate higher levels of total phenolic contents than young ones (Obsuwan *et al*., 2019). In the same way, the mature *Aranda* used in this study showed a high level of total phenolic content.

Interestingly, in the *A.* Bangkhuntian Gold, the total phenolic content was detected in a major proportion in the root part as 291.67 mg GAE/g extract, which is remarkably higher than other organs of another three cultivars, which have only 97.44-254.13 mg GAE/g extract (Table 1). Compared among the four plant parts, the floral bud and the blooming flowers have moderated equally levels of the total flavonoid and total phenolic contents ranging from 8.00-20.80 mg RUE/g extract and 97.44-143.44 mg GAE/g extract, respectively (Table 1).

In most of the *Aranda* hybrids, the highest ABTS and Fe (II) antioxidant activities were detected in the leaf part, with values ranging from 53.13 to 78.85% and 11.19 to 15.07%, respectively. These values were 1.13 to 2.78 times

higher than the antioxidant activities observed in the floral parts (Table 1). The DPPH activity of most *Aranda* hybrids was highest in the root part (67.37-80.90 %), which was 1.27-1.58 times higher than in the floral parts (Table 1). The ABTS and DPPH radical scavenging showed a positive correlation (r=0.762). Low levels of the Fe (II) antioxidant activity in this research indicated that the ethanolic extract of *Aranda* samples may not be favorable for this assay. Interestingly, as the total phenolic compounds are plant secondary metabolites for antioxidation, there was a strong correlation between the total phenolic contents and the ABTS antioxidant activity (r=0.90). Minh *et al*. (2016) reported that eleven phenolic compounds had been identified from *Phalaenopsis* hybrids such as protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, vanillin, ferulic acid, sinapic acid, p-coumaric acid, benzoic acid and ellagic acid. The strong correlation between the total phenolic content and total flavonoid content with the total antioxidant capacity was also reported in naked barley (Bechiri *et al*., 2024) and *Ajuga iva* (Lahrizi *et al*., 2024).

Although the four *Aranda* hybrids have diverse petal colors (pink, deep pink, yellow and deep yellow), there are minor differences in the total flavonoid, total phenolic contents and antioxidant activities among the variations in flowering shades. Therefore, the flowering stages (bud and blooming) or petal shade do not influence the total flavonoid and total phenolic contents and antioxidant activities of the *Aranda* hybrids. Among the *Aranda* hybrids, *A*. Bangkhuntian Gold exhibited the highest inhibitory effect on ABTS and DPPH radical scavenging in the root part (80.90-82.23%) (Fig 4-5), which correlates with the highest level of total phenolic contents in the roots. The richness of the total phenolic and antioxidant activities in the root of *A*. Bangkhuntian may be attributed to species specificity. *Aranda* is a monopodial orchid that grows upward, with a stem that is difficult to harvest for its floral inflorescences. Therefore, the orchid growers usually prune by cutting the top shoot of the aging *Aranda* to maintain a suitable level of plant height. Therefore, growers can benefit from the cut-off shoot, which consists of numerous leaves and aerial roots that can be used as a material for bio-substance extractions.

Phytochemical contents and antioxidant activities of *Aranda* after storage for six months

The dried powder of *Aranda* was kept at room temperature (25-28°C) or in a refrigerator (5-7°C) for six months to investigate the influence of long-term storage and temperature on the phytochemical contents and antioxidant capacities. The results showed that the total flavonoid content in the leaves and roots of all *Aranda* hybrids was sensitive to storage at room temperature, as the total flavonoid level significantly decreased under room temperature conditions (34.80-155.58 mg RUE/g extract)

Table 1: Antioxidant related property of *Aranda* hybrids at 0 month of storage.

Treatments	Total flavonoids (mg RUE/g extract)	Total phenolics (mg GAE/g extract)	ABTS (% inhibition)	DPPH (% inhibition)	Fe II chelating activity (% inhibition)
***Aranda* bangkhuntian gold**					
Flower buds	19.66cde	143.44cd	38.33f	63.75cd	2.71f
Blooming flowers	17.56def	123.26de	31.01g	51.18ef	9.89bcde
Leaves	29.10b	170.56c	66.79c	55.12de	11.19abc
Roots	11.25fg	291.67a	82.23a	80.90a	6.53cdef
***A.* Calypso**					
Flower buds	17.89def	101.96e	27.45g	40.44fg	6.81cdef
Blooming flowers	8.00g	123.79de	37.98f	48.10ef	6.03def
Leaves	40.05a	152.00cd	53.13d	51.58ef	11.19abc
Roots	20.46cd	143.67cd	46.07e	67.37bc	6.03def
***A.* Royal supply**					
Flower buds	20.80cd	97.44e	24.10g	47.49ef	2.63f
Blooming flowers	16.12def	101.22e	26.59g	34.01g	6.84cdef
Leaves	39.90a	254.13b	73.84bc	80.83a	12.75ab
Roots	12.41efg	221.00b	68.02c	74.47abc	5.52ef
***A.* Sayan duangporn**					
Flower buds	14.51def	101.00e	29.59g	43.11efg	5.98def
Blooming flowers	13.10def	121.70de	38.99f	48.01ef	10.70abcd
Leaves	26.63bc	229.65b	78.85ab	63.09cd	15.07a
Roots	17.01def	158.84cd	58.54d	75.81ab	7.27cdef

The data represent the mean of triplicate assay for each sample. The mean in the same column followed by the same letter are not significantly different at $P<0.05$.

compared to cool storage (71.83-241.49 mg RUE/g extract) (Fig 2). The study involved storing floral buds and blooming flower parts as storage samples for six months at either room or cool temperatures. The total flavonoid content in these samples ranged from 69.25-118.25 and 71.83-126.79 mg RUE/g extract, respectively. The results showed no significant difference in the flavonoid content at $P<0.05$ between the two storage conditions (Fig 1). Flavonoids are reported to be heat-sensitive substances. The rapid water loss during processing or prolonged exposure to sunlight of the samples can result in a decrease in the flavonoid content. In *Dryopteris erythrosora* leaf pretreatment by sunlight has a faster rate of water loss in dried samples, resulting in a marked reduction of total flavonoid level compared to shade drying (Zhang *et al*., 2019). In this study, the floral buds or blooming flowers have lower water content and the rate of water loss may be slower than in the leaves and the roots. Therefore, the flavonoid contents can be better maintained

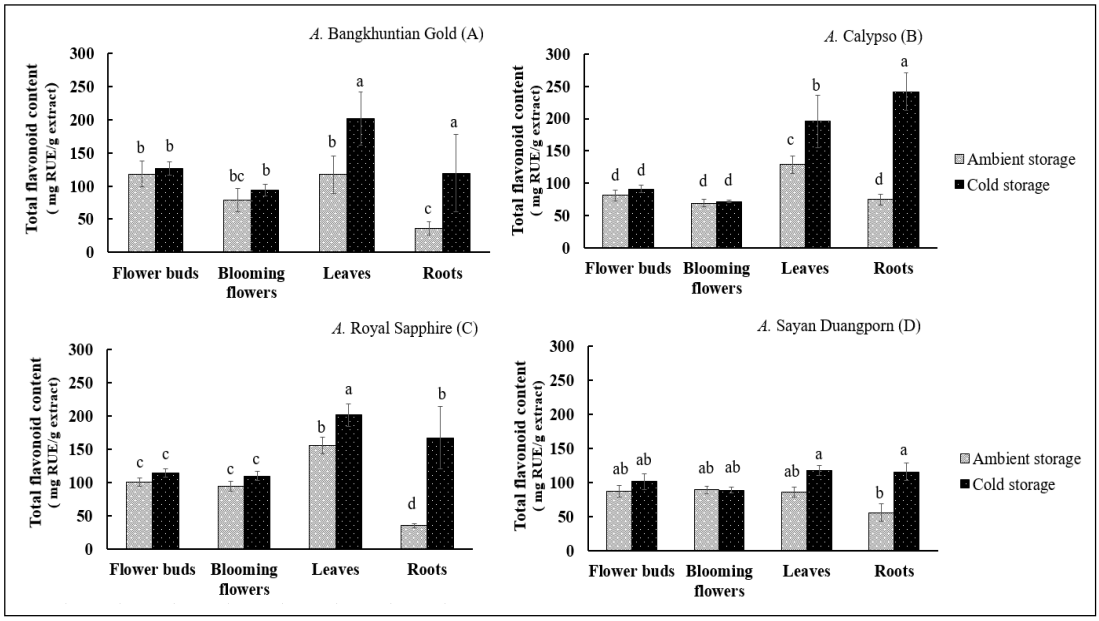


Fig 2: Total flavonoid contents in various parts of orchids stored at room temperature (25-28°C) or cool temperature (5-7°C) for six months.

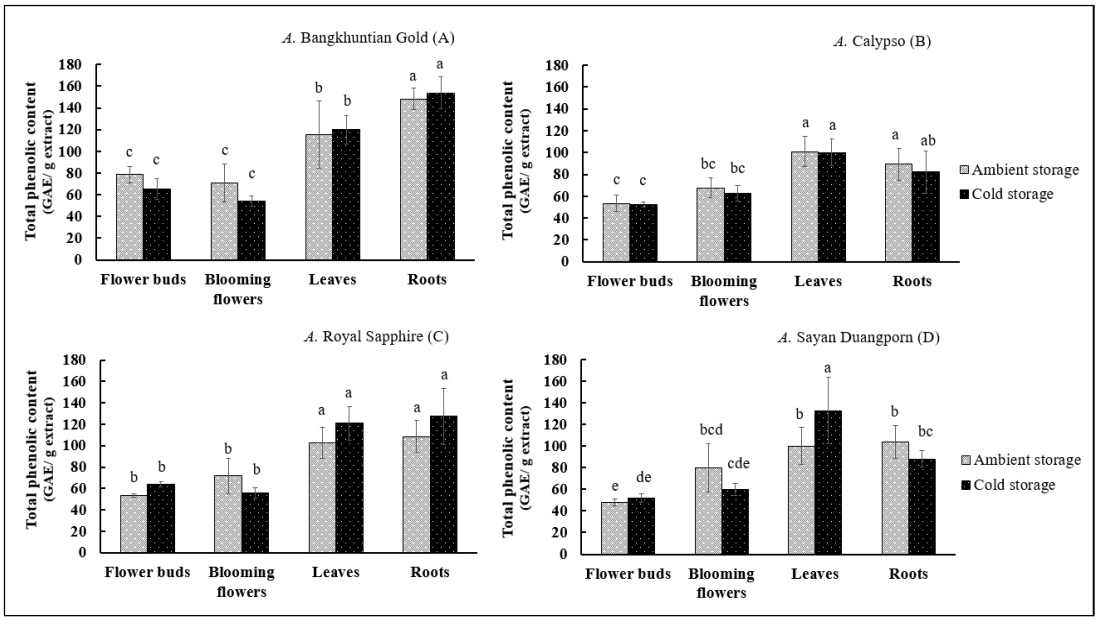


Fig 3: Total phenolic contents in various parts of orchids stored at room temperature (25-28°C) or cool temperature (5-7°C) for six months.

in long-term storage. The total phenolic contents from different parts of four *Aranda* hybrids were found to be at similar levels when stored under both conditions (Fig 3). It means that the room temperature has less influence on the total phenolic contents of the orchids. Similarly, the storage at room temperature or in a refrigerator had a non-significant influence on the ABTS, DPPH and Fe (II) radical scavenging of all parts of most *Aranda* hybrids (Fig 4-6), except for the *A.* Royal Sapphire stored in cool storage can maintain better ABTS activity in flower buds and blooming flowers (Fig 3).

The overall results suggested that the 25-28°C storage for six months has little affected the total phenolic contents and antioxidant activities compared to the cool storage because the dried samples lost their antioxidant properties slowly. Therefore, the dried sample can be kept at room temperature if the cool temperature facility is unavailable for at least six months.

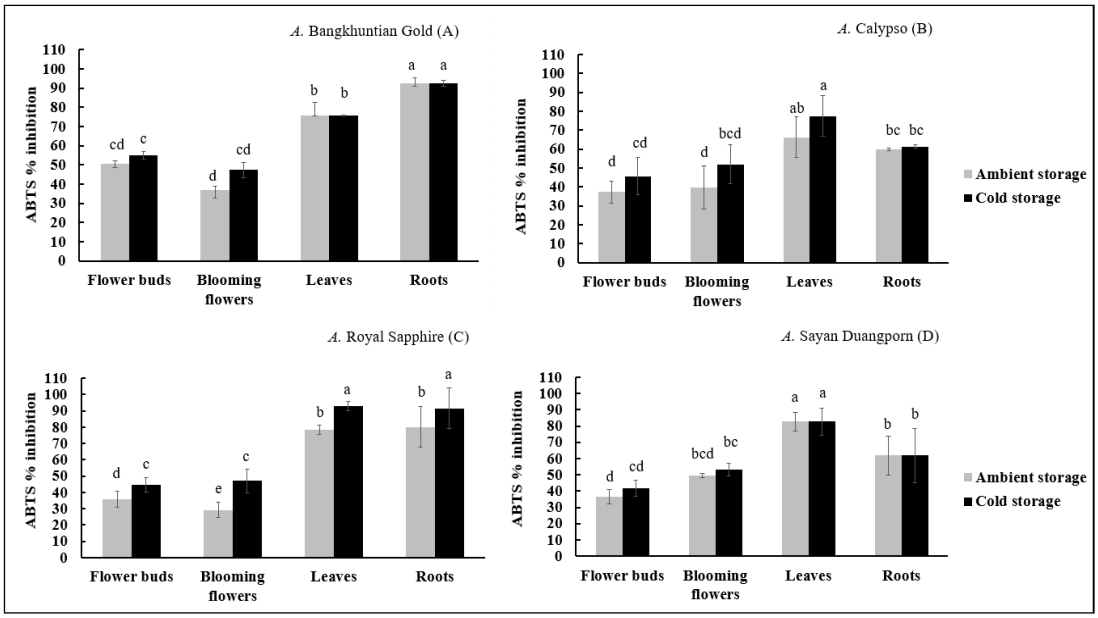
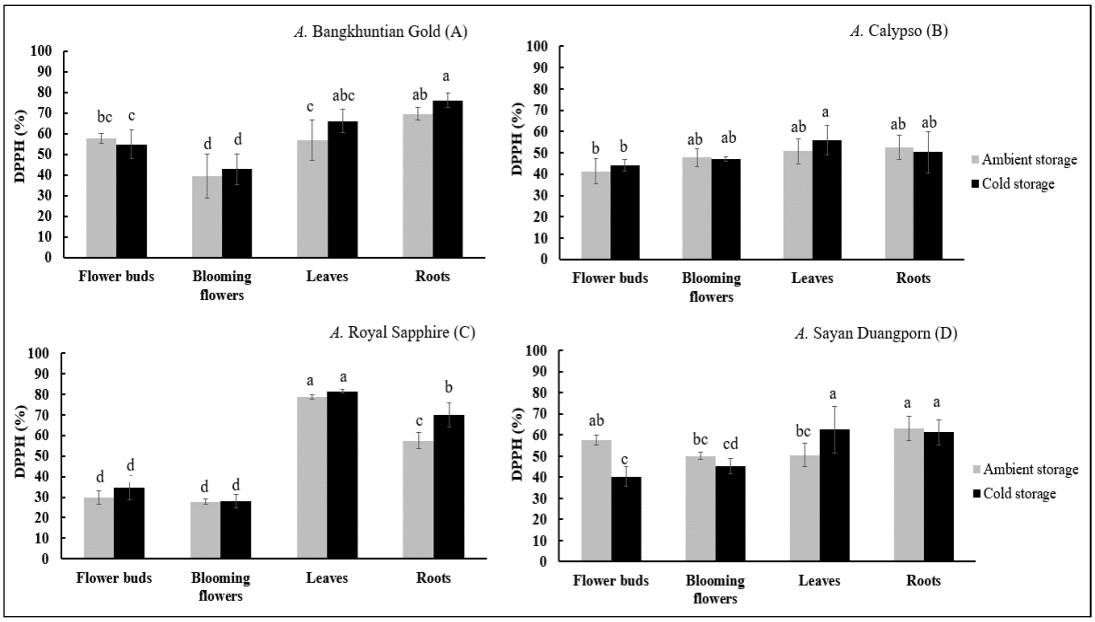


Fig 4: The ABTS antioxidant activity in various parts of orchids stored at room temperature (25-28°C) or cool temperature (5-7°C) for six months.



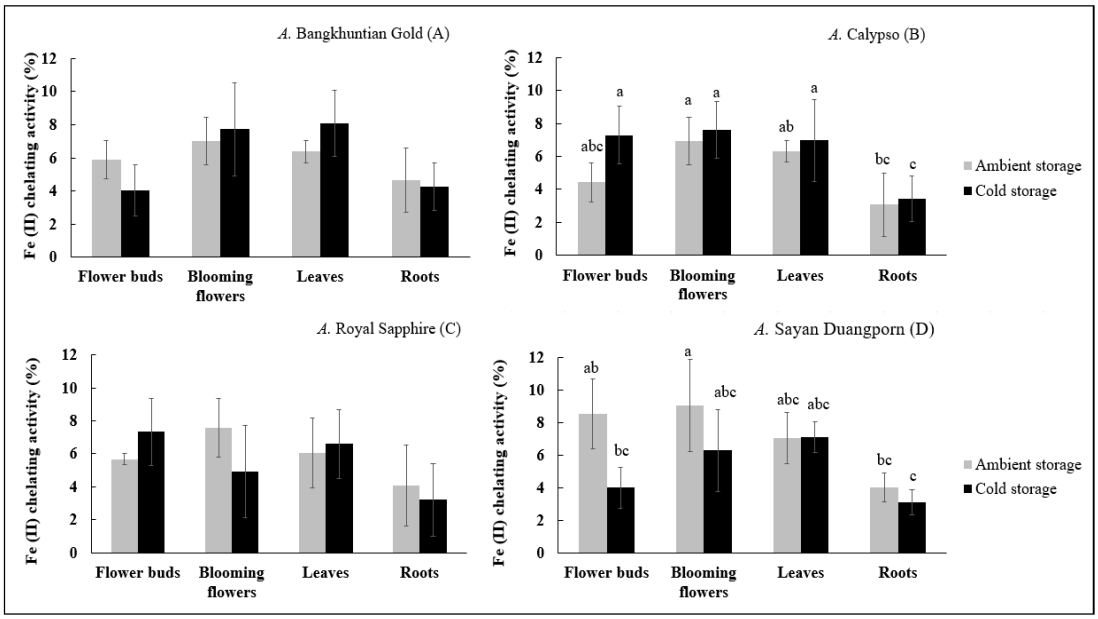


Fig 6: The Fe (II) antioxidant activity in various parts of orchids stored at room temperature (25-28°C) or cool temperature (5-7°C) for six months.

CONCLUSION

The four *Aranda* hybrids include *A.* Bangkhuntian Gold, *A.* Calypso, *A.* Royal Sapphire and *A.* Sayan Duangporn have the highest total flavonoids and phenolic contents, the ABTS and Fe (II) antioxidant activities in the leaves part, which are remarkably higher than the floral parts. However, the DPPH activity of most *Aranda* hybrids was the highest accumulated in the root part. Compared among the four *Aranda* hybrids, the *A.* Bangkhuntian Gold has the highest total phenolic contents in the leaf and ABTS and DPPH antioxidant activities in the root. The six months of dried samples storage at room temperature can maintain the total phenolic contents, the ABTS, the DPPH and Fe (II) antioxidant activities compared to the cool storage. However, the total flavonoid contents were decreased when stored under room temperature conditions.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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