Adv. Hort. Sci., 2025 39(2): 125-135
DOI: 10.36253/ahsc-16964
https://oaj.fupress.net/index.php/ahs

AHS
Advances in Horticultural Science

Phytochemical evaluation of selected *Phalaenopsis* cultivars

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Citation:
THATHSARANI Y.K.D.D., TIKIRIKUMARI J.A.T., PRIYADARSHAN A.I.S., HERATH H.M., SENANAYAKE S.P., 2025 - *Phytochemical evaluation of selected Phalaenopsis cultivars.* - Adv. Hort. Sci., 39(2): 125-135.

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Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no conflict of interests.

Received for publication 3 December 2024
Accepted for publication 31 March 2025

Key words: Anthocyanins, carotenoids, chlorophyll, cultivars, flavonoids, polyphenols.

Abstract: The genus *Phalaenopsis* in family Orchidaceae has gained popularity in the global floriculture market for its value as potted plants and cut flowers. Hybridization plays a pivotal role in breeding *Phalaenopsis,* enabling the development of novel cultivars with desirable traits such as diverse floral pigmentation patterns, enhanced longevity and improved growth rate. The selection of suitable parental cultivars is a crucial determinant in the hybridization process, as it plays a key role in defining pigmentation patterns and enhancing desirable traits for the development of superior cultivars. This research investigated the phytochemical composition, including carotenoids, anthocyanins, flavonoids and phenolics contents of selected commercial *Phalaenopsis* cultivars. Using UV-vis spectrophotometry, the chlorophylls, flavonoids, carotenoids, anthocyanins and phenolics contents in the flowers, leaves and roots of six cultivars were analyzed quantitatively. The results revealed significant variations in phytochemical properties across the tested cultivars and plant organs. *Phalaenopsis* cvs. Taipei Gold Gold Star, Red Lip 1770, Golden Sands Canary, Sogo Yukidian V3 and Queen Beer Mantefon exhibited promising phytochemical profiles. Notably, cvs. Taipei Gold Gold Star, Golden Sands Canary and Queen Beer Mantefon were identified as ideal parental candidates for hybridization due to their potential to develop distinctive floral colorations and robust vegetative traits. These findings provide valuable insights into the floral coloration and the phytochemical richness of vegetative parts in *Phalaenopsis* cultivars. This knowledge can contribute to the development of innovative, high-quality cultivars with enhanced survival rates and greater consumer appeal.

1. Introduction

The family Orchidaceae, one of the largest families of flowering plants, ranks second only to the family Asteraceae in its diversity and ecological prominence. Comprising over 736 genera, Orchidaceae has established a significant presence in the global floriculture market, valued for its use in both potted plants and cut flowers (Chase *et al.*, 2015). Among its commercially significant genera, *Phalaenopsis* stands out for its refined,

AHS - Firenze University Press
ISSN 1592-1573 (on line) - 0394-6169 (print)

elegant aesthetic and extended longevity, captivating both growers and consumers (Hsiao *et al.*, 2011). Hybridization serves as the primary breeding approach for *Phalaenopsis*, with intergeneric and interspecific hybridizations being the most commonly employed methods to develop novel cultivars. To date, 35,129 *Phalaenopsis* hybrids have been registered with the Royal Horticultural Society, reflecting the immense popularity of the genus (Hsu *et al.*, 2018). Dynamic and evolving consumer preferences have significantly driven hybridization efforts, making *Phalaenopsis* a focal point in modern orchid breeding.

The breeding objectives for *Phalaenopsis* focus primarily on morphology, color, and scent, with color being a key factor in shaping initial consumer impressions (Hsu *et al.*, 2018). With the increasing popularity and demand for orchids, commercial cultivars are developed with specific characteristics that enhance their large-scale applicability. Key traits include resilience, which reduces the need for inputs such as water, fertilizers, and pesticides—an essential factor given rising production costs and the growing emphasis on sustainability. Modern floriculture, a resource-intensive sector that utilizes energy, water, fertilizers, and propagation materials, faces the challenge of meeting market demands while minimizing environmental impact (Darras, 2020; Cardoso and Vendrame, 2022; Cardoso *et al.*, 2023; Bhardwaj *et al.*, 2024).

Phytochemicals play a crucial role in plant growth and development. While primary metabolites support physiological functions, secondary metabolites contribute significantly to plant's defense mechanisms (Thacker and Ram, 2020; Wani *et al.,* 2022). In orchids, various phytochemicals have been identified, serving both physiological and commercial purposes. Notable genera with economic significance include *Dendrobium* (He *et al.,* 2023), *Phalaenopsis* (Ling and Subramanium, 2007; Minh *et al.,* 2016), *Vanda*, *Bulbophyllum* (Lalrosangpuii and Lalrokimi, 2021), and *Cymbidium* (Axiotis *et al.,* 2021). Understanding the phytochemical composition of these orchids is essential for developing innovative, sustainable, and high-value cultivars that align with market trends and consumer preferences.

The vibrant coloration of *Phalaenopsis* hybrids arises from a complex mechanism of pigment accumulation. Flower color is primarily influenced by pigments such as chlorophylls, carotenoids, anthocyanins, and betalains. While many flowers derive their color from a single source of pigment, *Phalaenopsis* exhibits a broader palette through the combination of pigments. Yellow to orange shades are mainly due to the accumulation of carotenoids, whereas blue to red shades are typically attributed to anthocyanins (Hsu *et al.*, 2018). Combinations of purple anthocyanins and yellow carotenoids can result in perceived colors such as brown, bronze, and red in flowers (Lightbourn *et al.*, 2008). Hence, novel colors can be produced through hybridization, facilitating the combination of different pigments (Voegelpoel, 1990). Moreover, differential coloration can generate various pigmentation patterns such as blotches, stripes along veins, and irregular markings. These striking patterns and colors increase the aesthetic value of *Phalaenopsis* cultivars, capturing the attention of consumers and expanding their market appeal. The quality of the flower depends on the vegetative growth rate of the plant, as vigorous growth often correlates with superior flower characteristics. Photosynthesis, a primary physiological process, drives plant growth by converting light energy into chemical energy, enabling the synthesis of carbohydrates. Chlorophyll, the key pigment in photosynthesis, plays an essential role in this process. The two main forms of chlorophyll-chlorophyll a and chlorophyll b-absorb solar energy, facilitate carbon dioxide fixation, and convert energy into carbohydrates, which are vital for plant growth and development. Consequently, higher chlorophyll content is directly associated with improved photosynthetic efficiency and plant vigor. In addition to chlorophyll, phenolic compounds also play a crucial role in plant physiology and aesthetics. These compounds, including flavonoids such as anthocyanins, contribute significantly to plant defense mechanisms and pigmentation. Phenolic compounds accumulate as an adaptive response to environmental stress, highlighting their role in plant resilience (Lattanzio *et al.*, 2012).

In *Phalaenopsis* cultivars with desirable traits, flower color intensity is a key selection criterion in tissue culture and breeding programs (Ling and Subramaniam, 2007). However, the phytochemical profiles of *Phalaenopsis* cultivars remain largely fragmented, limiting the ability to develop hybrids with targeted traits. This lack of comprehensive data highlights the need for in-depth phytochemical studies to support informed breeding decisions. Therefore, this study aims to evaluate the phytochemical properties of selected *Phalaenopsis* cultivars, providing valuable insights for the development of high-value cultivars that align with market trends.

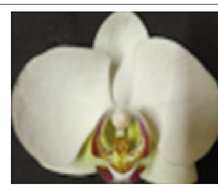
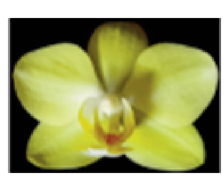
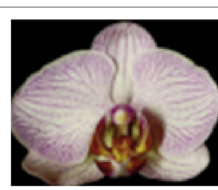
2. Materials and Methods

Plant materials

This study was conducted to evaluate phytochemical properties of selected *Phalaenopsis* cultivars.

Six commercially available *Phalaenopsis* cultivars with different color combinations were obtained from the growers. The details of the selected six commercial *Phalaenopsis* cultivars are presented in Table 1. The plants were maintained in the plant house of the Department of Plant and Molecular Biology, University of Kelaniya, Sri Lanka. The fresh, full-bloomed flowers, young leaves (the leaf nearest to the flower spike) and roots of each cultivar were collected in the morning and used for phytochemical analysis. The roots and leaves were cleaned with distilled water and wiped. All the samples were frozen at -80°C immediately after collection. The frozen samples were grounded using mortar and

Table 1 - List of selected commercial Phalaenopsis cultivars

No.	Name		Reference for the cultivar name	Abbreviation
1	*Phalaenopsis* cv. Red Lip 1770		Locally produced cultivar	RL1770
2	*Phalaenopsis* cv. Golden Sands Canary		(Lee *et al.,* 2020)	GSC
3	*Phalaenopsis* cv. Sogo Yukidian V3		(Lee *et al.,* 2020)	SYV3
4	*Phalaenopsis* cv. Queen Beer Mantefon		(Lee *et al.,* 2020)	QBM
5	*Phalaenopsis* cv. Taipei Gold Gold Star		(Lee *et al.,* 2020)	TGGS
6	*Phalaenopsis* cv. Brother Strips		(Lee *et al.,* 2020)	BS

pestle. The weights of each powdered plant material were measured and quantified to obtain a standard weight.

Determination of chlorophyll and carotenoid contents

Chlorophyll and carotenoid contents of leaves, roots and flowers were measured using the methods described by Nguyen *et al.* (2018) with slight modifications. Known volume of dried powder was mixed with a known volume of 80% acetone and kept at 4°C overnight. The mixture was centrifuged at 13,000 g for 5 min to obtain the supernatant. The supernatant was tested to determine the absorbance of chlorophyll *a*, chlorophyll *b* and carotenoids in 80% acetone at 664 nm, 647nm and 441 nm respectively using the UV- visible spectrophotometer (Thermo Fisher Scientific, Vantaa, Finland). Concentrations of chlorophyll *a,* chlorophyll *b* and carotenoids were calculated using the following equations (Porra, 2002).

$$\text{Chlorophyll a} = (12.25 \times \text{Absorbance}_{664}) - (2.55 \times \text{Absorbance}_{647}) \times \text{volume of the supernatant (ml)/sample weight (g)}$$

$$\text{Chlorophyll b} = (20.31 \times \text{Absorbance}_{647}) - (4.91 \times \text{Absorbance}_{664}) \times \text{volume of the supernatant (ml)/sample weight (g)}$$

$$\text{Carotenoids} = (4.69 \times \text{Absorbance}_{441} \times \text{volume of the supernatant (ml)/sample weight (g)}) - 0.267\ (\text{chlorophyll a} \pm \text{b})$$

$$\text{Total chlorophyll} = \text{Chlorophyll a} \pm \text{Chlorophyll b}$$

Determination of anthocyanin content

A known weight of dry powder of leaves, roots and flowers were mixed with a known volume of acidified methanol (99% methanol containing 1% HCl). The mixture was incubated for 24 hours at room temperature followed by centrifugation at 4°C and 3000 rpm for 5 minutes. The obtained supernatant was then subjected to measure the absorbance at 530 nm and 657 nm on the UV-visible spectrophotometer (Thermo Fisher Scientific, Vantaa, Finland). Concentration of anthocyanins were calculated using the following equation.

$$\text{Anthocyanin content} = (\text{Absorbance}_{530} - 0.33 \times \text{Absorbance}_{657} / 31.6) \times \text{volume of supernatant (ml) / sample weight (g)}$$

Determination of total flavonoid content

The known weight of dry powder of leaves, roots and flowers were mixed with methanol and incubated for 24 hours at room temperature. The supernatant was obtained by centrifugation at 4°C and 3000 rpm for 5 minutes. Equal volumes of the supernatant were mixed with 2% Aluminum chloride. The mixture was stirred and kept for 15 minutes. The absorbance was measured at 430 nm using the UV-visible spectrophotometer (Thermo Fisher Scientific, Vantaa, Finland). Quercetin was used as the reference standard. The flavonoid content of each plant part was expressed as milligrams of Quercetin equivalents of grams of dry weight (mg QE/g DW) using the following equation.

$$C = C_1\ \text{(Volume of the supernatant (ml))/(Dry weight (g))}$$

Where C is total flavonoid content of the extract and C_1 the quercetin concentration (mg/ml).

Determination of total polyphenol content

The acidified methanol extracts were prepared using the same procedure done for the anthocyanin content analysis. 100 µl of the extract was mixed with 2 ml of 7.5 % Na_2CO_3 and allowed to equilibrate for 2 minutes. After 2 minutes diluted Folin-Ciocalteu reagent was added (1:10 v/v). The absorbance was measured at 765 nm using the UV- visible spectrophotometer (Thermo Fisher Scientific, Vantaa, Finland). Gallic acid was used as the reference standard. The polyphenol content of each plant part was expressed as milligrams of Gallic acid equivalents of grams of dry weight (mg GAE/g DW) using the following equation.

$$C = C_1\ \text{(Volume of the supernatant (ml))/(Dry weight (g))}$$

Where C is the total polyphenol content of the extract and C_1 th gallic acid concentration (mg/ml).

Statistical analysis

All the experiments were conducted in triplicates (n=3). The data were expressed as mean ± SD (standard deviation). An analysis of variance (ANOVA) test was performed with Tukey's Honest Significant Difference test (HSD) at $p \leq 0.05$ using R software version 4.3.2.

3. Results

Chlorophyll content

Figure 1 illustrates the chlorophyll a, chlorophyll b, total chlorophyll (a + b), and the chlorophyll a/b ratio in extracts from different parts (leaves, roots, and flowers) of six *Phalaenopsis* cultivars. The analysis revealed that the concentrations of chlorophyll a and b were significantly higher in the

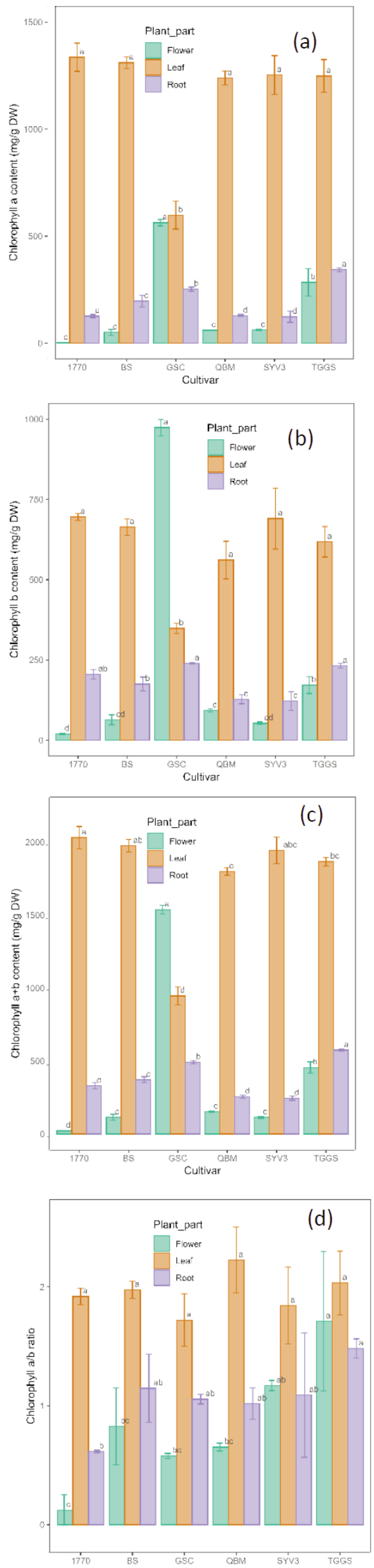


Fig. 1 - (a) Chlorophyll a, (b) chlorophyll b, (c) chlorophyll a+b contents and (d) chlorophyll ratio in extracts from different plant organs of the six *Phalaenopsis* cultivars tested. BS= *Phalaenopsis* cv. Brother Strips; GSC= *Phalaenopsis* cv. Golden Sands Canary; QBM= *Phalaenopsis* cv. Queen Beer Mantefon; RL1770= *Phalaenopsis* cv. Red Lip 1770; SYV3= *Phalaenopsis* cv. Sogo Yukidian V3.

leaves compared to the roots and flowers across all tested cultivars. Similarly, the total chlorophyll (a + b) content in the leaves was higher than in the roots of the cultivars. Additionally, the leaves of all cultivars displayed a higher chlorophyll a/b ratio than that observed in the roots (Fig. 1). In this study, the leaves and roots of all tested cultivars, except for the roots of cv. Red Lip 1770 (0.62±0.01), had a chlorophyll a/b ratio higher than 1. Therefore, in most of the tested cultivars, the chlorophyll a/b ratio in leaf and root extracts was below 2.0, while in a few cases, it was approximately 2.0. These findings indicate the predominance of chlorophyll a over chlorophyll b in both leaves and roots, reflecting their physiological roles in photosynthesis. The analysis of chlorophyll content among the tested *Phalaenopsis* cultivars revealed significant variations in both leaf and root tissues.

The cv. Red Lip 1770 exhibited the highest levels of chlorophyll a (1335.81±65.94 mg g^{-1} DW) and chlorophyll b (695.80±11.00 mg g^{-1} DW) in the leaves, yielding the highest total chlorophyll content (chlorophyll a + b) of 2031.61±75.83 mg g^{-1} DW. Conversely, cv. Golden Sands Canary reported the lowest levels of chlorophyll a (598.02±65.01 mg g^{-1} DW) and chlorophyll b (348.59±16.20 mg g^{-1} DW), resulting in the lowest total chlorophyll content of 946.60±61.35 mg g^{-1} DW.

Chlorophyll content in root extracts varied significantly among cultivars. Cv. Taipei Gold Gold Star had the highest chlorophyll a content (343.53±8.96 mg g^{-1} DW), while cv. Golden Sands Canary exhibited the highest chlorophyll b content (239.67±1.99 mg g^{-1} DW). In contrast, cv. Sogo Yukidian V3 showed the lowest levels of both chlorophyll a (123.49±26.32 mg g^{-1} DW) and chlorophyll b (122.17 ± 28.63 mg g^{-1} DW).

Total chlorophyll content in roots followed a similar trend, with cv. Taipei Gold Gold Star achieving the highest value (575.53±5.11 mg g^{-1} DW), while cv. Sogo Yukidian V3 exhibited the lowest (245.66±14.44 mg g^{-1} DW).

Among all the examined *Phalaenopsis* flower samples, cv. Golden Sands Canary exhibited the highest chlorophyll *a* (563.16±14.80 mg g^{-1} DW) and chlorophyll *b* (973.66±25.26 mg g^{-1} DW) contents. As a result, its total chlorophyll (chlorophyll *a+b*) content was also the highest in *Phalaenopsis* cv. Golden Sands Canary (1536.82±29.49 mg g^{-1} DW). Conversely, cv. Red Lip 1770 had the lowest chlorophyll *a* (2.18±2.10 mg g^{-1} DW) and chlorophyll *b* (19.70±2.83 mg g^{-1} DW) contents, resulting in the lowest total chlorophyll (*a*+*b*) content among all tested flowers. Additionally, all flower samples exhibited a chlorophyll *a*/*b* ratio of less than 1, except for cv. Taipei Gold Gold Star (1.70±0.56) and cv. Sogo Yukidian V3 (1.18±0.21).

Total flavonoid content

Figure 2 illustrates the total flavonoid content in extracts from various parts of *Phalaenopsis* cultivars, highlighting significant variation in flavonoid distribution within the same cultivar. Specifically, flower extracts contained significantly higher total flavonoid levels compared to other plant parts. Notably, the highest total flavonoid content was observed in cv. Brother Strips (0.42±0.0 mg QE g^{-1} DW) whereas cv. Queen Beer Mantefon exhibited the lowest (0.19±0.00 mg QE g^{-1} DW). On the other hand, root extracts, of cv. Queen Beer Mantefon contained significantly higher levels of flavonoids (0.114±0.04 mg QE.g^{-1} DW) compared to cv. Golden Sands Canary, which had the lowest value (0.057±0.01 mg QE g^{-1} DW).

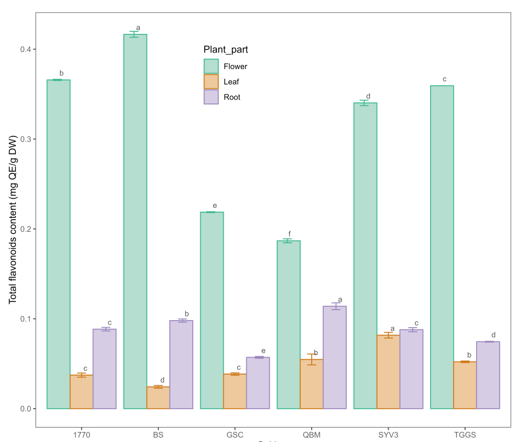


Fig. 2 - Total flavonoid contents in extracts from different plant organs of the six *Phalaenopsis* cultivars tested. BS= *Phalaenopsis* cv. Brother Strips; GSC= *Phalaenopsis* cv. Golden Sands Canary; QBM= *Phalaenopsis* cv. Queen Beer Mantefon; RL1770= *Phalaenopsis* cv. Red Lip 1770; SYV3= *Phalaenopsis* cv. Sogo Yukidian V3; TGGS= *Phalaenopsis* cv. Taipei Gold Gold Star.

Interestingly, both cv. Sogo Yukidian V3 and cv. Red Lip 1770 reported equal total flavonoid levels in root extracts (0.088±0.02 mg QE g^{-1} DW). Moreover, leaf extracts of cv. Sogo Yukidian V3 exhibited the highest flavonoid content (0.082±0.00 mg QE·g^{-1} DW), while cv. Brother Strips had the lowest (0.024±0.00 mg QE·g^{-1} DW). These findings highlight the significant variation in flavonoid distribution across different plant organs of the same *Phalaenopsis* cultivar.

Total polyphenol content

Figure 3 depicts the total phenolics content in the leaves, flowers and roots of different *Phalaenopsis* cultivars, revealing substantial variation across plant organs within the same cultivar and among different cultivars. Flower sample from cv. Golden Sands Canary exhibited the highest phenolics content (10.58±0.109 mg.GAE g^{-1} DW) whereas those from cv. Sogo Yukidian V3 reported the lowest (1.60±0.01 mg.GAE g^{-1} DW). Leaf extracts from cv. Golden Sands Canary again showed the highest phenolics content (6.55±0.49 mg.GAE g^{-1} DW) whereas cv. Brother Strips had the lowest (1.75±0.01 mg.GAE g^{-1} DW). Similarly, root extracts from cv. Taipei Gold Gold Star were rich in phenolics (5.68±0.95 mg.GAE g^{-1} DW) whereas the lowest was *Phalaenopsis* cv. Brother Strips recorded the lowest amount (0.45±0.32 mg.GAE g^{-1} DW).

Anthocyanin and carotenoid contents

Anthocyanin and carotenoid contents in different plant parts of *Phalaenopsis* cultivars are shown in figures 4 (a) and 4 (b), respectively. Among the tested cultivars, the flower extracts of cv. Queen Beer Mantefon exhibited the highest anthocyanin content

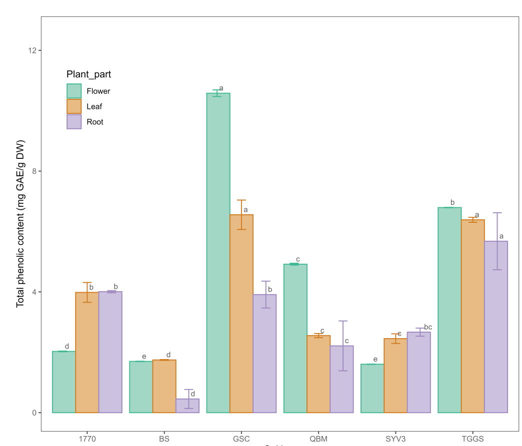


Fig. 3 - Total phenolic contents in different plant organs of the six *Phalaenopsis* cultivars tested. BS= *Phalaenopsis* cv. Brother Strips; GSC= *Phalaenopsis* cv. Golden Sands Canary; QBM= *Phalaenopsis* cv. Queen Beer Mantefon; RL1770= *Phalaenopsis* cv. Red Lip 1770; SYV3= *Phalaenopsis* cv. Sogo Yukidian V3; TGGS= *Phalaenopsis* cv. Taipei Gold Gold Star.

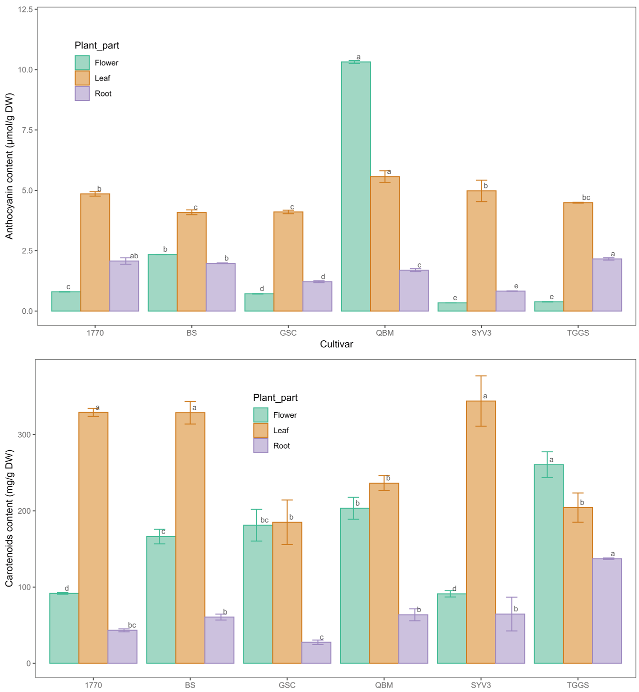


Fig. 4 - The anthocyanin contents (a) and the carotenoids contents (b) in different plant organs of the six *Phalaenopsis* cultivars tested. BS= *Phalaenopsis* cv. Brother Strips; GSC= *Phalaenopsis* cv. Golden Sands Canary; QBM= *Phalaenopsis* cv. Queen Beer Mantefon; RL1770= *Phalaenopsis* cv. Red Lip 1770; SYV3= *Phalaenopsis* cv. Sogo Yukidian V3; TGGS= *Phalaenopsis* cv. Taipei Gold Gold Star.

(10.32±0.06 µmol g^{-1} DW), significantly surpassing all other cultivars. Interestingly, this cultivar had the lowest flavonoid content in flower extracts. In contrast, cv. Sogo Yukidian V3 showed the lowest anthocyanin content among the flower extracts (0.34±0.00 µmol g^{-1} DW). Although completely yellow cultivars such as Taipei Gold Gold Star showed no visible anthocyanin-related pigmentation, small amounts of anthocyanins were detected (0.38±0.01 µmol.g^{-1} DW). Conversely, this cultivar displayed the highest carotenoid content in its flower extracts (260.50±16.97 mg.g^{-1} DW), significantly exceeding those of the other tested cultivars. Flower extracts from cultivars with white tepals and colored labellum such as Red Lip 1770 and Sogo Yukidian V3, displayed lower contents of both anthocyanins and carotenoids. Furthermore, cv. Queen Beer Mantefon reported significantly higher content of both anthocyanins and carotenoids simultaneously, a pattern not observed in other cultivars. The yellow coloration of the flowers varied across cultivars, with Brother Strips displaying yellow only in the labellum, while Golden Sands Canary showing yellow pigmentation throughout the entire flower. Despite these color differences, there was considerable variation in both anthocyanin and carotenoid contents among the flower extracts of the tested cultivars. Root extracts generally displayed the lowest anthocyanin content among the tested plant organs.

The root extracts of cv. Taipei Gold Gold Star exhibited the highest anthocyanin content (2.16 ± 0.05 µmol/g DW), while cv. Sogo Yukidian V3 showed the lowest (0.83±0.01 µmol/g DW). In contrast, leaf extracts displayed more variability, with cv. Queen Beer Mantefon having the highest anthocyanin content (5.57±0.23 µmol/g DW), while cv. Brother Strips contained the lowest (4.09±0.10 µmol/g DW).

The carotenoid content was generally higher in leaf extracts compared to other plant organs, with the exception of cv. Taipei Gold Gold Star. In this cv, carotenoid levels were 204.27±19.19 mg.g^{-1} DW in leaves and 260.50±16.97 mg.g^{-1} DW in flowers. Root extracts consistently displayed lower carotenoid contents compared to other plant parts across all cultivars. Among leaf extracts, cv. Sogo Yukidian V3 exhibited the highest carotenoid content (344.05 ± 32.95 mg g^{-1} DW), whereas cv. Golden Sands Canary reported the lowest (184.99±29.29 mg.g^{-1} DW).

The highest carotenoid content was found in root extracts from cv. Taipei Gold Gold Star (137.19 ± 1.07 mg/g DW), while the lowest was in those from cv. Golden Sands Canary (27.61±2.92 mg/g DW). Overall, both anthocyanin and carotenoid levels exhibited significant variation across the different cultivars and plant organs.

4. Discussion and Conclusions

The genus *Phalaenopsis* within the family Orchidaceae holds significant prominence in the floriculture industry due to its prolonged blooming, captivating aesthetic, ease of cultivation, adaptability and its success in hybridization. Hybridization offers breeders a unique opportunity to develop hybrids with enhanced physiological traits and diverse flower coloration. However, achieving such advancements relies heavily on the selection of parental cultivars with desirable traits, making this step important for creating novel cultivars.

Breeding strategies, plant tissue culture, and biotechnological advancements play a crucial role in developing novel traits and expanding the global commercialization of orchids (Tiwari *et al.,* 2024). Understanding the specific phytochemical properties of different plant parts, both floral and vegetative, in *Phalaenopsis* orchids provides valuable insights for informed cultivar selection, ultimately contributing to the development of resilient, high-quality, and commercially viable hybrids.

Comprehensive knowledge of anthocyanin and carotenoid contents in leaves, roots, and flowers provides insights into pigmentation, which is closely linked to flower coloration, as well as physiological responses such as light absorption and stress tolerance. These insights can enhance breeding efficiency and ensure targeted improvements in hybrid cultivars. This approach aligns with the findings of Nguyen *et al.* (2018), emphasizing the importance of exploring the phytochemical composition of orchids to unlock their full potential for breeding and cultivation. By leveraging such information, breeders can innovate more effectively and meet the growing demand for unique and visually appealing *Phalaenopsis* cultivars in the global market.

The chlorophyll content analysis of *Phalaenopsis* cultivars revealed that leaves exhibited significantly higher chlorophyll levels than roots, a pattern consistent with findings by Trelka *et al.* (2010). This is expected, as leaves serve as the primary site for photosynthesis in plants. Additionally, the chlorophyll *a*/*b* ratio was higher in leaves compared to roots, supporting observations by Martin *et al.* (2010), who reported that epiphytic orchids generally have higher chlorophyll *a*/*b* ratios in leaves than in roots. Notably, the chlorophyll *a*/*b* ratios in epiphytes are typically low, often around 2.0 or less.

In this study, the chlorophyll *a*/*b* ratios in leaves and roots of all tested cultivars, except the roots of Red Lip 1770 exceeded 1.0. While several ratios were less than 2.0, most were approximately 2.0, which aligns with the characteristic shade adaptation of epiphytic orchids. A reduced chlorophyll *a*/*b* ratio, as observed in previous orchid studies, suggests a functional adaptation to low-light environments, allowing these plants to efficiently capture and utilize available light.

The results obtained in this study further confirm the shade-adaptive traits of *Phalaenopsis* cultivars. Understanding these adaptations enhances the knowledge of their physiological responses and could support targeted breeding and cultivation strategies to optimize growth under various light conditions.

Higher chlorophylls content serves as a valuable indirect indicator of enhanced photosynthetic efficiency of the plant (Lin and Hsu, 2004). Among the tested cultivars, Red Lip 1770 exhibited the highest chlorophyll *a*, chlorophyll *b* and total chlorophyll levels in its leaf extracts. This suggests that Red Lip 1770 may have superior photosynthetic efficiency, particularly under shade conditions, making this trait critical during its vegetative phase. Interestingly, significant variations in chlorophyll content were observed in the roots. Notably, the cultivars with the highest chlorophyll content in their leaf extracts were not the same as those exhibiting the highest chlorophyll content in their roots. This divergence indicates a broader variability in chlorophyll distribution between leaves and roots across the tested cultivars. *Phalaenopsis* cv. Golden Sands Canary demonstrated the highest chlorophyll content in its flower extracts among the tested cultivars. These findings highlight the differential accumulation of chlorophylls among *Phalaenopsis* cultivars, which could be utilized for selecting superior parental lines in breeding programs.

However, flowers generally reported lower chlorophyll content compared to leaves and roots. This difference could be attributed to the degradation of chlorophyll or the reduced activity of chlorophyll-synthesizing enzymes in flowers. A similar phenomenon has been observed in carnations, where petals contain significant amounts of chlorophyll during early development, which declines as the flower matures (Nurcahyani *et al.,* 2021).

The presence of chlorophyll in flowers is essential for carbohydrate synthesis during their development, supporting energy requirements and metabolic processes. For *Phalaenopsis,* investigating chlorophyll content at different stages of flower development is essential to gain a deeper understanding of its role and dynamics. Such studies could provide insights into optimizing flowering conditions and improving overall plant health and productivity.

Significant variations in carotenoids and anthocyanin contents were observed in the flowers of studied *Phalaenopsis* cultivars. Cultivars with purple flowers such as Queen Beer Mantefon exhibited the highest levels of both anthocyanin and carotenoid contents indicating that these pigments contribute synergistically to the flower's coloration.

In contrast, yellow-flowered cultivars like Taipei Gold Gold Star contained anthocyanins in minimal quantities, with carotenoids serving as the dominant pigments. Notably, flowers with white petals and yellow labellum, such as Sogo Yukidian V3, displayed lower carotenoid content compared to entirely yellow flowers.

Interestingly, an inverse relationship was observed between flower and leaf pigment content with cultivars displaying low anthocyanin and carotenoid levels in their flowers while exhibiting higher levels in their leaves. This suggests a potential redistribution or differential regulation of pigment synthesis in different plant parts. Therefore, a wide variation in distribution of carotenoid and anthocyanin pigments was observed in different plant parts of the same cultivar. Furthermore, some flowers exhibited visible coloration that did not align with their measured pigment content, highlighting the complexity of pigment interactions. As reported by Narbona *et al.* (2021), variations in the type or ratio of pigments can influence flower color, while changes in pigment concentration primarily affect color intensity.

The results also underline the intricate patterns and color variations in *Phalaenopsis* flowers, which range from simple monochromatic tones to complex patterns. Such variability is typical of orchids and other ornamental species like irises and crowfoots. This study provides valuable insights into the pigment composition and color diversity in *Phalaenopsis* flowers, enhancing our understanding of their aesthetic and physiological characteristics.

Among the different plant parts analyzed, roots exhibited the highest flavonoid content, while flowers showed the lowest. Flavonoids, known for their multifunctionality, play important roles in various physiological and ecological processes. In epiphytic orchids, such as Phalaenopsis, the velamen radicum is vital for water and nutrient absorption, storage, and UV-B protection. Flavonoids are also integral to leaves and other epidermal tissues, providing photoprotection and antioxidant properties that safeguard plants from environmental stressors (Nguyen *et al.*, 2018). In flowers, flavonoids serve as UV-B protectants and as attractants for pollinators, facilitating successful pollination. Interestingly, cultivars with white petals, such as Red Lip 1770 and Sogo Yukidian V3, exhibited higher flavonoid content despite having minimal anthocyanin and carotenoid levels. This is consistent with findings that white petals, which reflect all wavelengths of visible light, often contain high concentrations of UV-absorbing flavonoids like flavones and flavonols (Narbona *et al.*, 2021). The significant variations in flavonoid content among cultivars may emphasize the potential of selecting cultivars with high flavonoid levels in specific plant parts as parental materials for breeding programs. A study on flowers, leaves, and stems of White Clover cultivars revealed the potential for selecting cultivars with targeted concentrations of flavonoids (Carlsen *et al.*, 2008). Hence, applying a similar selection approach in Phalaenopsis cultivars could enhance desirable traits, including stress tolerance and aesthetic appeal. Plant phenolics, a class of secondary metabolites, play a major role in mitigating oxidative stress by acting as strong antioxidant agents (Trelka *et al.*, 2010). By neutralizing reactive oxygen species (ROS), phenolics help minimize oxidative damage in plants, contributing to improved resilience and longevity. Additionally, phenolics are involved in delaying senescence, thereby enhancing the functional and aesthetic lifespan of plant organs, as highlighted by Cavaiuolo *et al.* (2013).

In this study, Golden Sands Canary exhibited the highest polyphenol content in both leaves and flowers, indicating its superior capacity to combat oxidative stress. Conversely, BS showed the lowest polyphenol levels in leaf and root extracts, suggesting a comparatively weaker antioxidant defense. These differences suggest that polyphenol content is a critical determinant of a plant's ability to endure oxidative stress, which, in turn, influences its storage life and performance as a potted plant or cut flower.

Selecting and breeding cultivars with high phenolics content could enhance stress tolerance and longevity in ornamental plants. Such efforts would not only improve the plants' defensive mechanisms but also potentially extend their market value and usability. Orchid varieties have high phenolics and flavonoids compounds and have higher restraint power of free radicals. Moreover, a relationship can be observed between phytochemicals and morphological traits (Ebrahimi *et al.,* 2020). The findings underscore the importance of incorporating antioxidant-related traits into breeding programs to produce robust and resilient *Phalaenopsis* cultivars with superior oxidative stress management capacities.

Breeding efforts for *Phalaenopsis* have largely focused on producing white, pink, and red hybrids, leading to market saturation. Orchid breeders are now shifting their focus toward developing flowers with unique pigmentation patterns distributed across various regions of the flower (Hsu *et al.,* 2018). The findings of this study highlighted the potential of leveraging cultivars with differential pigment accumulation to achieve this goal. For instance, combining anthocyanin- and carotenoid-rich cultivars such as *Phalaenopsis* cv. Queen Beer Mantefon with white cultivars like Red Lip 1770 and Sogo Yukidian V3 could result in novel hybrids with diverse and striking pigmentation patterns. The creation of novel flower coloration patterns is critical for maintaining consumer interest in *Phalaenopsis* orchids, as aesthetic appeal remains a key factor driving market demand. Among the tested cultivars, Queen Beer Mantefon stood out for its high concentrations of both anthocyanin and carotenoids, while *Phalaenopsis* cv. Brother Strips and Golden Sands Canary reported the highest flavonoid and polyphenol contents, respectively. These traits indicate potential resilience and extended storage life, adding further value to these cultivars as breeding materials. Additionally, the study revealed significant variation in chlorophyll, flavonoid, and polyphenol content across the vegetative parts of different cultivars. These traits are equally important for breeding robust plants with enhanced stress tolerance, longevity, and overall vigor.

In conclusion, this study provides insights into floral pigmentation and phytochemical composition of vegetative organs, facilitating the identification and selection of desirable traits for *Phalaenopsis* breeding programs. By selecting parental materials based on pigment profiles and vegetative characteristics, breeders can develop new cultivars with enhanced aesthetic appeal and improved physiological performance. Such advancements will not only enhance the ornamental value of *Phalaenopsis* orchids but also strengthen their adaptability and commercial potential. Despite these findings, the relationship between phytochemicals and the physiological traits in commercial *Phalaenopsis* cultivars remains unexplored. Among the studied commercial cultivars, Taipei Gold Gold Star, Golden Sands Canary and Queen Beer Mantefon can be recommended as potential parental candidates for hybridization due to their distinctive floral colorations and robust vegetative traits for adaptability.

Acknowledgements

Authors wish to acknowledge the National Agricultural Research Policy for providing financial assistance for this research under the grant No: NARP/21/UK/SC/01.

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