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Biochemical Analyses of *Phalaenopsis violacea* Orchids

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Abstract: Biochemical analyses were carried out on the leaves and flower samples of *Phalaenopsis violacea* orchid plants. Anthocyanins and anthocyanidins, chlorophylls, phenolics, proteins and sugar contents were used to analyse and provide the general idea regarding partial selection of superior qualities for tissue culture and plant breeding purposes. Through the biochemical and statistical analyses, the *Phalaenopsis violacea* orchid plant No. 9 has been found to be the most superior plant among all the 12 different samples of orchid plants in terms of its biological activities. The anthocyanidins contents detected were as follow: cyanidin ($11.53 \pm 0.07 \mu\text{g mL}^{-1}$), delphinidin ($12.73 \pm 0.08 \mu\text{g mL}^{-1}$), malvidin ($7.65 \pm 0.05 \mu\text{g mL}^{-1}$), pelargonidin ($8.98 \pm 0.06 \mu\text{g mL}^{-1}$), peonidin ($21.24 \pm 0.13 \mu\text{g mL}^{-1}$) and petunidin ($117.12 \pm 0.69 \mu\text{g mL}^{-1}$). The total amount of chlorophyll contents (chlorophyll a and b) was $8.97 \pm 0.1 \mu\text{g mL}^{-1}$ while the chlorophyll concentration was $448.67 \pm 5.1 \mu\text{g g}^{-1}$. As for the phenolic and protein contents estimation, the results obtained were 1.78 ± 0 and $55.00 \pm 4.15 \mu\text{g}$, respectively. In soluble sugar analysis, a relatively smaller amount of sugar content was detected for about $0.19 \pm 0 \mu\text{mol}$ in the orchid plant No. 9. The main objectives of this study were selecting partial superior qualities of *Phalaenopsis violacea* orchid plant for tissue culture and plant breeding purposes through different biochemical analyses.

Key words: Biochemical analyses, *Phalaenopsis violacea* orchid, quality

INTRODUCTION

Phalaenopsis is one of the orchid species that is known for its graceful, arching spray of beautiful flowers. The flowers appear like butterflies with its wings outstretched (Fighetti, 2006). In Indonesia, the *Phalaenopsis* is known as *Anggerik bulan* or the moon orchid, possibly comparing the flower to the romantic full moon (Fighetti, 2006).

Phalaenopsis orchid species are popular because they are relatively inexpensive, easy to grow and very rewarding. One of the *Phalaenopsis* species that involved in this research was *Phalaenopsis violacea* (Fig. 1). *Phalaenopsis violacea* is found mostly in Peninsular Malaysia, Borneo and Sumatra. This flower of the Borneo type is bigger, rounder and more valued compared with the Malay and Sumatra types. Generally, *Phalaenopsis violacea* grows well if the plant attached to pieces of wood or tree trunk, tree ferns and coconut husks.

The two ultimate objectives of this study were to firstly determine and distinguish on how to select for the superior qualities of *Phalaenopsis violacea* orchid plant for tissue culture and plant breeding purposes through different biochemical analyses. The second main objective was to study and determine the main anthocyanidin that gives the most pigment colour to the flower of the *Phalaenopsis violacea*. The other objectives included as to analyse and estimate the amount of different anthocyanidins, chlorophylls, phenolics, proteins and sugar contents that present in the flowers and leaves of *Phalaenopsis violacea* from 12 different orchid plant samples being provided.

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Fig. 1: *Phalaenopsis violacea* plant. The yellow bar (-) in the bottom left side of flower image represents 6 mm in actual size

MATERIALS AND METHODS

Collection of Plant Materials

Phalaenopsis violacea orchid flowers and leaves were used as the plant materials. These plants were placed in the AIMST glasshouse (24°C) throughout the research and were taken care to ensure and maintain the healthy growth of the orchid plants during each of the experiment conducted in these studies.

The fresh full-bloomed of the *Phalaenopsis violacea* orchid flowers and their young leaves (the leaves that appeared on top of the rest of the orchid leaves and nearest to the orchid flower spike) were collected in the morning and kept in the icebox (cool condition) before continue for any further analysis. The fresh weights of each flower and leaf were measured and quantified to become the standard weight for the whole biochemical analyses of *Phalaenopsis violacea* orchid plants (the standard weight being used in this research studies was 0.5 g for all the flower and leaf samples). The leaves sample being used for all the biochemical analyses were selected only at the distal part of the whole young leaves (around 4-5 cm from the distal part of the leaf) for samples weighing.

All the biochemical analyses were done on the same day or a day after the samples were being collected. This was to prevent the degradation of the samples (flower and leaf) quality especially the chlorophyll and anthocyanidins pigments that will affect the results obtained later in the biochemical analyses.

Anthocyanins and Anthocyanidins Analysis

Extraction of Flower Pigments

For the pigment extraction procedures, the collected fresh petals from the full-bloomed flowers were weighed for 0.5 g. The cooled extract was washed twice with absolute diethyl ether solution. The diethyl ether layers were discarded while the remaining aqueous layer was heated at 80°C using water bath for 3 min.

Determination of Anthocyanins and Anthocyanidins Contents Using UV Spectrophotometer

The presence of anthocyanins and anthocyanidins were determined by the absorption peaks at 505 nm wavelengths. The amount of anthocyanidins ($\mu\text{g mL}^{-1}$) that presence in each flower samples was determined by using the standard calibration curve of the 6 main anthocyanidins (cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin) for better estimation results. Presence of these six anthocyanidins has been carried by using thin layer chromatography (TLC) method (unpublished results).

Chlorophylls Determination

For the extraction of chlorophyll samples, 0.5 g of fresh orchid leaves from each plant samples were grounded with 1.0 g of calcium carbonate (CaCO_3) powder and 5 mL of 80% acetone solution at 4°C using chilled mortar and pestle (in the ice box). Finally the extraction volumes were added up to 25 mL using volumetric flask with 80% acetone solution. The absorbance readings were measured at 646 and 663 nm using the spectrophotometer. The chlorophyll contents ($\mu\text{g mL}^{-1}$) were calculated using Harborne (1973) method.

Phenolics Analysis

The concentration of total phenols in the extracts were determined by using Folin Ciocalteu reagent and the external calibration curve with caffeic acid was used for phenolic estimation in mg. In this analysis, 0.1 mL of the crude extract (obtained from the 0.5 g of protein leaves extraction in the protein analysis, which will be discussed later) for each plant samples was subjected to 50 times dilution by adding 4.9 mL of extraction solution (protein extraction buffer). The absorbance readings were measured at 760 nm (Singleton *et al.*, 1999).

Proteins Analysis

The extraction of crude protein was conducted at 4°C using 0.5 g of fresh leaves from each plant samples and was grounded by chilled mortar and pestle with 10 mL of protein extraction buffer. The cellular materials were removed by centrifugation at 12000 rpm for 20 min at 4°C. The supernatant was collected for the next step of protein analysis. About 0.1 mL of the cellular extract was used, substituting the amount of BSA (as in the optimization of standard curve samples preparation) before adding the protein reagent (Bradford, 1976).

Sugar Analysis

As for the sugar extract samples, 0.5 g of the fresh full-bloomed flower was used. The absorbance reading was measured at 510 nm wavelengths for each sample for 3 times using spectrophotometer and recorded (Somogyi-Nelson, 1952).

Statistical Analyses

The data obtained from all the biochemical analyses were analyzed using One-Way ANOVA and the differences contrasted using the Duncan's Multiple Range Test. All these statistical analysis were performed at the significance level of 5% using SPSS 13.0 software (SPSS Inc., USA).

RESULTS AND DISCUSSION

Anthocyanins and Anthocyanidins Contents

The Fig. 2 showed the different anthocyanidins concentration being detected in 4 different *Phalaenopsis violacea* orchid plants (plant No. 8-11). Among the 4 orchid plants, plant No. 9 contained the highest amount of anthocyanidins content. The amounts of pigments detected within the orchid flower plant No. 9 were as follow: $11.53 \pm 0.07 \mu\text{g mL}^{-1}$ of cyanidin, $12.73 \pm 0.08 \mu\text{g mL}^{-1}$ of delphinidin, $7.65 \pm 0.05 \mu\text{g mL}^{-1}$ of malvidin, $8.98 \pm 0.06 \mu\text{g mL}^{-1}$ of pelargonidin, $21.24 \pm 0.13 \mu\text{g mL}^{-1}$ of peonidin and $117.12 \pm 0.69 \mu\text{g mL}^{-1}$ of petunidin (Table 1). The total anthocyanins content was $1792.50 \mu\text{g g}^{-1}$ (or 1.79 mg g^{-1}) and obtained by multiplying the extraction volume (5 mL of 2M HCl) with the total concentration of anthocyanidins ($\mu\text{g mL}^{-1}$) per gram of fresh weight (of the flower sample).

The second highest amount of anthocyanidins content was in plant No. 11. The amounts of anthocyanidin pigments detected within this plant sample were approximately similar as in plant No. 9. The contents were as follow: $11.38 \pm 0.16 \mu\text{g mL}^{-1}$ of cyanidin, $12.57 \pm 0.18 \mu\text{g mL}^{-1}$ of delphinidin, $7.54 \pm 0.12 \mu\text{g mL}^{-1}$ of malvidin, $8.84 \pm 0.15 \mu\text{g mL}^{-1}$ of pelargonidin, $20.97 \pm 0.29 \mu\text{g mL}^{-1}$ of peonidin and $115.63 \pm 1.57 \mu\text{g mL}^{-1}$ of petunidin (Table 1). The total anthocyanins content was $1769.30 \mu\text{g g}^{-1}$ (or 1.77 mg g^{-1}). Among all the anthocyanidin pigments, petunidin contributed the highest amount of colour concentration to the orchid flower of *Phalaenopsis violacea* plants while malvidin was least present pigment in all the flower samples being tested (Table 1).

According to Farzad *et al.* (2002), within the cell of the flower plant, the co-pigments and anthocyanins presence in the vacuole as the chemical complex that held together by the hydrophobic interactions which were under the genetic control. The strength of the interaction bonds within the chemical complex will determine the flower colour. The anthocyanidins (anthocyanins without sugars attached) will provide the basis for floral pigmentation but flowers with same anthocyanidin can have different colours depending on the identity and the concentration of the co-pigments and also the pH of the vacuole.

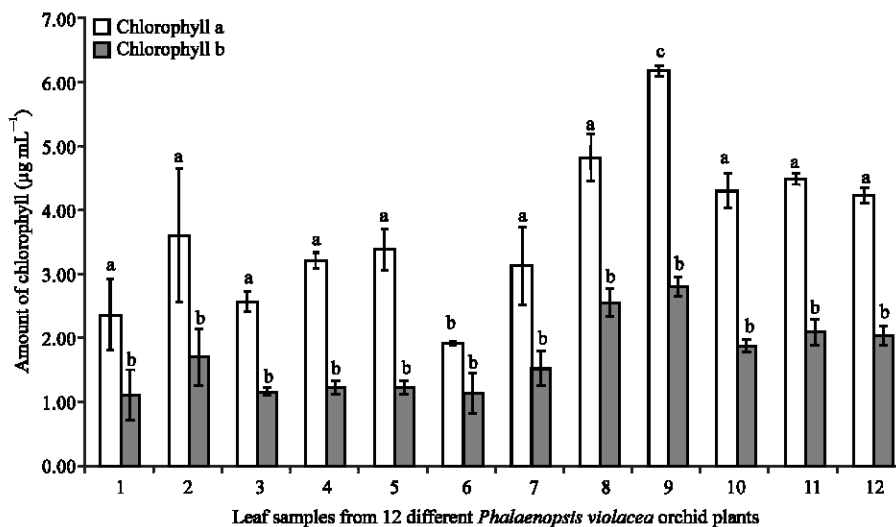


Fig. 2: The amount of chlorophylls content detected in 12 different *Phalaenopsis violacea* orchid plants. Data were analysed using one-way ANOVA and the differences contrasted using Duncan's multiple range test. Different letter(s) (a, b and c) indicate the values are significantly different ($p < 0.05$). The values were mean \pm SD

Table 1: The overall results obtained from the biochemical analyses of the 12 different samples of *Phalaenopsis violacea* orchid plants

Plant No. (flower /leaf samples)	Anthocyanidins content ($\mu\text{g mL}^{-1}$)							Chlorophyll content				
	Protein (μg)	Phenolic (μg)	Sugar (μmol)	Cyanidin	Delphinidin	Malvidin	Pelargonidin	Peonidin	Petunidin	Total chlorophyll ($\mu\text{g mL}^{-1}$)	Chlorophyll concentration ($\mu\text{g g}^{-1}$)	
1	43.33±4.13a	1.94±0.00a	ND	ND	ND	ND	ND	ND	ND	3.46±0.94	172.92±46.84	
2	53.00±5.76b	2.03±0.01a	ND	ND	ND	ND	ND	ND	ND	5.30±1.48	264.75±74.04	
3	30.00±4.73c	2.35±0.03b	ND	ND	ND	ND	ND	ND	ND	3.71±0.10	185.33±41.60	
4	25.33±3.50c	1.75±0.01c	0.34±0.01a	ND	ND	ND	ND	ND	ND	4.44±0.18	221.75±9.150	
5	47.67±4.08a	2.02±0.01a	0.32±0.00a	ND	ND	ND	ND	ND	ND	4.60±0.43	229.75±21.48	
6	35.00±3.03c	1.67±0.01c	0.33±0.00a	ND	ND	ND	ND	ND	ND	3.05±0.28	152.42±14.09	
7	55.67±4.27b	2.03±0.01a	0.22±0.00b	ND	ND	ND	ND	ND	ND	4.63±0.88	231.42±43.93	
8	45.67±5.43a	1.97±0.01a	0.24±0.00b	7.01±0.190a	7.69±0.210a	4.38±0.14a	4.87±0.17a	12.81±0.34a	71.83±1.86b	7.37±0.26	368.50±12.85	
9	55.00±4.15b	1.78±0.00a	0.19±0.00b	11.53±0.07a	12.73±0.08a	7.65±0.05a	8.98±0.06a	21.24±0.13a	117.12±0.69b	8.97±0.10	448.67±5.150	
10	41.00±3.29c	1.77±0.02a	0.22±0.00b	9.62±0.09a	10.61±0.10a	6.27±0.07a	7.24±0.09a	17.69±0.17a	98.05±0.90b	6.16±0.29	307.92±14.63	
11	60.00±3.35b	2.07±0.01a	0.22±0.00b	11.38±0.16a	12.57±0.18a	7.54±0.12a	8.84±0.15a	20.97±0.29a	115.63±1.57b	6.58±0.20	329.00±10.02	
12	70.00±4.38a	2.08±0.00a	ND	ND	ND	ND	ND	ND	ND	6.26±0.20	312.92±9.790	

*The values were the mean±SD, ND: Not determined, — Among the highest results obtained, — Among the lowest results obtained, Different letter(s) (a and b) indicate the values are significantly different ($p < 0.05$)

In most orchid cultivars, especially in hybrid orchids, the highest flower colour intensity for a particular species was one of the main criteria being selected to perform the tissue culture and orchid breeding techniques among the other orchid plant of the same species. In this biochemical analysis, the highest amounts of anthocyanidins content were found within the *Phalaenopsis violacea* orchid plant No. 9.

Chlorophylls Content

Chlorophyll was the pigment responsible for the green colour production in plant leaves. There were 2 types of chlorophyll being studied in this analysis: chlorophyll a and chlorophyll b. Chlorophyll a was the main pigment that assists the photosynthesis reaction while chlorophyll b was the accessory pigment that passes the energy absorbed through the visible wavelength to chlorophyll a for photosynthesis process. Chlorophyll a can be detected at the wavelength of around 662 nm while chlorophyll b at the wavelength of around 644 nm (Lisiewska *et al.*, 2006). Figures showed the chlorophyll contents and concentration obtained from the experiment conducted.

From the Fig. 2, the highest amount of chlorophylls content being detected derived from the *Phalaenopsis violacea* orchid plant No. 9 and followed by the orchid plant No. 8. The amount of chlorophyll a and chlorophyll b being detected in the leaves of orchid Plant No. 9 were 6.18 ± 0.08 and $2.80 \pm 0.15 \mu\text{g mL}^{-1}$, respectively with the ratio of 2.2 (ratio of chl a: chl b). While in orchid plant No. 8, the amount of chlorophyll a was $4.82 \pm 0.37 \mu\text{g mL}^{-1}$ and chlorophyll b was $2.55 \pm 0.22 \mu\text{g mL}^{-1}$ with the ratio of 1.9. Among all the 12 orchid plant samples, orchid plant No. 6 had the lowest chlorophylls content of chlorophyll a ($1.92 \pm 0.03 \mu\text{g mL}^{-1}$) and chlorophyll b ($1.13 \pm 0.31 \mu\text{g mL}^{-1}$) with the ratio of 1.7

Chlorophyll a concentration was observed to be higher than chlorophyll b concentration in all the 12 different *Phalaenopsis violacea* orchid plant samples. According to Lahai *et al.* (2003), chlorophyll a:b ratio was lesser in the plants that were grown under the shade area than in plants that were exposed to the full sunlight. In other words, *Phalaenopsis violacea* orchid plants can grow well in the shaded area but with the sufficient sunlight exposure in order to grow well at the maximum level as the ratio of chlorophyll a:b was relatively high as being obtained in the result for all the orchid plant samples. When they compared with chlorophyll a, chlorophyll b was increased significantly under low light exposure. They also suggested that chlorophyll b concentration may increase under the stressful conditions compared to chlorophyll a (Lahai *et al.*, 2003). We believed that chlorophyll loss is associated to environment stress and the variation in total chlorophyll as a good indicator of stress in orchid generally.

As in the total chlorophyll concentration, orchid plant No. 9 was the highest ones contributing $448.67 \pm 5.15 \mu\text{g g}^{-1}$, followed by the orchid plant No. 8 with the amount of $368.50 \pm 12.85 \mu\text{g g}^{-1}$. The least amount of total chlorophyll concentration was found in orchid plant No. 6 with $152.42 \pm 14.09 \mu\text{g g}^{-1}$ (Table 1). The higher the amount of chlorophylls content and concentration, the better quality of that orchid plant was from the other orchid plant samples in the same species.

In this chlorophyll analysis, orchid plant No. 9 had the best quality in terms of its highest chlorophylls content and concentration that indirectly showed the plant has the most efficient photosynthesis mechanism among the 12 orchid plant samples provided. All the chlorophyll extraction works were performed in the dim light environment and sometimes the test tubes that contain the chlorophyll extraction were covered with the aluminum foils in order to minimize the degradation and isomerization of chlorophylls. The addition of anhydrous calcium carbonate (CaCO_3) solution during the grinding process was to adjust the pH of the sample extraction to pH 8-9 while preventing the possibility for the conversion of chlorophyll pigments to pheophytin pigments (Bellomo and Fallico, 2007).

Chlorophylls extraction and separation were extremely susceptible to number of chemical transformations which affect the low yields and lack of separation of chlorophylls a and b. Thus there has been a method known as counter-current chromatography being developed for the better isolation of chlorophyll contents (Jubert and Bailey, 2006).

Phenolics Content

Phenolic compounds in orchid plant leaves were involved in several physiological mechanisms such as UV-protecting agents in plant tissues and involved in plant-pathogen interactions, both constitutively and as newly induced compounds. Generally, the amount of phenolic compounds being synthesized by the plant depends on the UV-light exposure, environment temperature, nutrition provided to the plant and the genetic factors of the plant itself (Andreotti *et al.*, 2006).

The phenolic compounds accumulate in different plant tissues and cells during the plant growth and under the influence of various environmental stimuli, respectively (Hutzler *et al.*, 1998; Maisuthisakul *et al.*, 2007; Stavrianakou *et al.*, 2006). There was a significant part in many plants where the phenolic substances were secreted on the leaf surface by the specialized tissues, either secretory glands or glandular hairs (Stavrianakou *et al.*, 2006).

The amounts of phenolics content among all the 12 orchid plant were relatively similar to one and other samples (1.8-2.4 μg of caffeic acid) (Table 1). However, from the Table 1, it showed that relatively higher amount of phenolics content has been detected in the orchid plant No. 3 (2.35 \pm 0.03 μg), followed by orchid plant No. 11 (2.07 \pm 0.01 μg) and orchid plant No. 12 (2.08 \pm 0 μg). The least amount of phenolic content was being detected in the orchid plant No. 6 (1.67 \pm 0.01 μg). Depends on the conditions of the orchid plant, sometimes the higher amount of phenolics content in a plant signified the better quality of the plant itself. This was due to the reason that if the plant was able to contain and secrete larger amount of this antioxidant compound (phenolic acid), the plant was considered to be in better condition in terms of its defence mechanism towards pathogen attacks.

The variability of the phenolic compounds and compositions detected in the leaves was considered as the prerequisite for their role in defence mechanisms (Andreotti *et al.*, 2006). In this phenolic analysis, the largest amount of phenolic content being detected among the 12 different *Phalaenopsis violacea* orchid plant samples was orchid plant No. 3. Secreted phenolic compounds also protected the leaf surfaces against the phytopathogenic microorganisms and had allelopathic properties by inhibiting or stimulating the growth of neighbouring plants if the compounds leached to the nearby soil (Stavrianakou *et al.*, 2006).

The concentration of the leaf phenolic compounds can be influenced by the ontogenetic ages (indicated by the position of the leaves along the shoots being formed), wounding effects and environmental conditions especially UV-light radiation. Mechanical injuries from pruning had been shown to induce the accumulation of phenolic compounds during the defence mechanism towards the injured plant tissues (Andreotti *et al.*, 2006).

Even though Folin-Ciocalteu method was a rapid and widely-used assay to investigate the total phenolic compounds but it was also known that different phenolic compounds had different responses in the Folin-Ciocalteu method. The total phenolic content differed among the different types and parts of the plants (Maisuthisakul *et al.*, 2007). Therefore, in this research study, the standard graph of caffeic acid in units of μg was used to calculate the total amount of phenolic content that present in all the orchid plant samples.

Another reason for the accumulation of these phenolic substances was due to restriction in plant growth when the food resources limitation occurred. Under this condition, the plant would react by converting more carbon sources and transported them to defensive structures and also to the production of carbon-based secondary metabolites such as phenolics (Stavrianakou *et al.*, 2006).

Therefore, for the better understanding of the ecological functions of phenolic compounds, it was necessary to study and know the chemical structure of the interested compounds, their biosynthesis pathways and regulations as well as the tissue localization where these phenolics can be found abundantly (Hutzler *et al.*, 1998). In one research paper, it reported that there has been observation on the correlations between phenolic content and the root formation in the *in vitro* culture or cuttings of many plant species (Schnablová *et al.*, 2006).

Proteins Content

Proteins were consisted of a large percentage of the plant cell and helped to carry out many different cell functions in the plant mechanism. In plants, protein synthesis occurred in 3 different parts of subcellular compartments: the cytoplasm, plastids and mitochondria and each part contained different protein synthetic machinery (Buchanan *et al.*, 2000).

The highest amount of protein content being detected in orchid leaves were in orchid plant No. 12 and then followed by orchid plant No. 11 (Table 1). Total of $70.00 \pm 4.38 \mu\text{g}$ of protein content was found in orchid plant No. 12 and in orchid plant No. 11, the amount of protein content obtained from the experiment was $60.00 \pm 3.35 \mu\text{g}$. Orchid plant No. 4 had the least amount of protein content being detected, that was around $25.33 \pm 3.50 \mu\text{g}$ from all the orchid plant samples (Table 1).

Since protein content was very important in a plant system, so the higher amount of the protein content in a particular orchid plant, the higher amount of amino acids accumulation and the better the plant quality would be in comparison with the other orchid plant samples. Therefore, orchid plant No. 12 was considered as the best quality orchid plant in terms of protein content in the leaves among all the 12 different *Phalaenopsis violacea* orchid plant samples provided.

Protein synthesis was very important especially in cell growth, cell differentiation and cell reproduction in plants. About 20% of the protein was located in the photosynthetically active cell, for example the young mesophyll cell which can be found in the leaf structure (Buchanan *et al.*, 2000).

Sugar Contents

Sugar or carbon-derived sources in plants were mainly constituted from the carbohydrate food resources. Sugar can be derived from the various groups of the carbohydrate that can be stored and reserved in different parts of the plant through photosynthesis process for the maintenance of growth and survival of the plants.

Amount of sugar content detected through the sugar analysis and estimation of glucose standard curve seemed to be divided into two main ranges of concentration (the first group was more than $0.30 \mu\text{mol}$ [$>0.30 \mu\text{mol}$] of glucose content and the second was less than $0.30 \mu\text{mol}$ [$<0.30 \mu\text{mol}$] of glucose content) (Table 1). The orchid plant No. 1, 2 and 3 seemed to be among the highest group with the ranges from 0.34 ± 0.01 , 0.32 ± 0 and $0.33 \pm 0 \mu\text{mol}$, respectively compared with the rest of the 5 different *Phalaenopsis violacea* orchid plant samples provided. The lowest amount of sugar content being detected was $0.19 \pm 0 \mu\text{mol}$ from the orchid plant No. 9.

For the comparison of the best quality of full-bloomed flower, the amount of sugar content (sucrose or any monosaccharide sugar) must be higher than the rest of the other full-bloomed flower samples. This was because the larger amount of sugar content presents in the flower, the faster the spiking and flower emergence was observed. The sugar content was found to be an important nutrient that significantly contributed its part for the flower synthesis and blooming (Kataoka *et al.*, 2004; Sood *et al.*, 2006).

The content of reducing sugar seemed to be considerably lower in younger flower petals but increased rapidly as the flower stages reached to the maximum full bloom. The sugars in the flower development may be multifunctional as they can act as energy source, osmotic regulators and as the precursors for metabolic processes (Sood *et al.*, 2006). There has been a study performed that

suggested the close correlation between photosynthesis and flower induction and development in *Phalaenopsis* orchid where the accumulation of carbohydrates (sugar and carbon source) due to the photosynthetic process may play an important role in flower induction together with other factors such as high temperature, light intensity and carbon dioxide (CO₂) enrichment on sugar contents (Kataoka *et al.*, 2004). In angiosperms such as rose plants, carbohydrate used to support flowering which obtained through photosynthesis (McLaughlin *et al.*, 2000).

Summary of the Biochemical Analyses in 12 Different *Phalaenopsis* *Violacea* Orchid Plant Samples

By comparatively using the biochemical and statistical analyses, the *Phalaenopsis violacea* orchid plant No. 9 had the most superior qualities among all the 12 different samples of orchid plant (Table 1). This orchid plant has been detected with the highest amount of anthocyanins and anthocyanidins contents (11.53±0.07 µg mL⁻¹ of cyanidin, 12.73±0.08 µg mL⁻¹ of delphinidin, 7.65±0.05 µg mL⁻¹ of malvidin, 8.98±0.06 µg mL⁻¹ of pelargonidin, 21.24±0.13 µg mL⁻¹ of peonidin and 117.12±0.69 µg mL⁻¹ of petunidin) and also the highest amount of chlorophyll contents (chlorophyll a was 6.18±0.08 µg mL⁻¹ and chlorophyll b was 2.80±0.15 µg mL⁻¹) as well as the chlorophyll concentration (448.67±5.15 µg g⁻¹). In addition, the orchid plant No. 9 also contained relatively high amount of phenolic (1.78±0 µg), protein (55.00±4.15 µg) and sugar (0.19±0 µmol) contents. All the biochemical analyses were important in determining which *Phalaenopsis violacea* orchid plant samples was superior than the rest of the 12 orchid plant samples within the same species that were being provided.

CONCLUSION

Biochemical analyses were carried out to determine the quality of the particular *Phalaenopsis violacea* orchid in order to choose the best plant for micropropagation in future. The amounts of chlorophyll, phenolic, protein and sugar contents obtained were varied from one plant to another plant. Such variation can be due to the factors like environmental temperature, sunlight exposure, genetic variation, pathogen and fungal attacks and the availability of nutrients provided. The main anthocyanidin detected in the flower of *Phalaenopsis violacea* orchid was petunidin pigment that usually contributes to the bluish-red colour to the flowers. As the results from this research, *Phalaenopsis violacea* orchid plant No. 9 has been selected as the most superior plant when compared with all the 12 different samples of orchid plant being provided. Highest amount of anthocyanins and anthocyanidins contents and also the highest amount of chlorophyll contents (chlorophyll a and chlorophyll b) as well as the chlorophyll concentration made this *Phalaenopsis violacea* orchid plant No. 9 being chosen as the best plant among the other orchid plant samples. The orchid plant No. 9 also contained relatively high amount of phenolic, protein and sugar contents (Table 1). All the biochemical analyses were important in determining which *Phalaenopsis violacea* orchid plant samples was superior than the rest of the 12 orchid plant samples within the same species that were being provided. Further studies and analyses must be done through other different modern methods such as High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), Mass Spectrometry (MS) and Nuclear Magnetic Resonance (NMR) as the preliminary tests on determining the superior quality of the *Phalaenopsis violacea* orchid plants before proceed into the tissue culture and breeding purposes.

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