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Analysis of bioactive compounds, polysaccharides and antioxidant activity in different parts of *Dendrobium* 'Sonia Jo Daeng'

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ABSTRACT

Numbers of research studies have revealed the potency of bioactive substances from *Dendrobium* spp. especially in East Asia. However, only limited information on detected bioactive compounds from the Thai *Dendrobium* hybrids, therefore, this research aimed to analyze the amount of some bioactive compounds, polysaccharides, and antioxidant activity from leaves, stems, roots, flowers and peduncles of 1- and 4-year-old *Dendrobium* 'Sonia Jo Daeng'. The studies showed that the highest total phenolic compounds were found in the flowers of 1-year-old plants (7.23±0.80 mg GAE/g DW) followed by roots, flowers and leaves of 4-year-old plants (6.79±1.88, 6.63±0.67, and 6.07±0.62 mg GAE/g, respectively). The maximum anthocyanin level was found in the flowers of 1- and 4-year-old plants (13.28±2.09 and 10.21±6.35 mg/l, respectively). The polysaccharide was highest in flowers of 1-year-old plants (153.95±12.63 mg glucose/g polysaccharide). The highest antioxidant activity was found in the flowers of 1-year-old plants (6.12±0.52 mg trolox/g DW) as well as in roots of 4-year-old plants (5.89±1.31 mg trolox/g DW). The results indicated that antioxidant activities were related to amounts of total phenolic compounds, contents of anthocyanin and polysaccharides.

Keywords: anthocyanin; antioxidant; orchid flower; phenolic; phytochemicals; tropical orchid

1. INTRODUCTION

Herbal medicine has played a critical role in disease treatment process and was the basis of most early medical treatments. Since, many disorder symptoms generated by reactive oxygen species (ROS), which occurred by regular metabolic processes caused injury to human cells. Nowadays, aging society and healthy food are also currently interesting topics for the current global trends. Phenolic compounds are known bioactive substances, which are of the main interest in functional and pharmaceutical industries due to their multifunctional roles in curing several depressive diseases and aging correlated unhealthiness. Thus, herbal products including nutritional additives, medicinal and balance diets are believed to be fruitful in defending peoples confronted to ROS damages and sickness such as inflammatory, cancer, cardiovascular and deteriorating illness with small amount of side effects to the human body (Huang et al., 2005; Jo et al., 2012; Zengin et al., 2015). *Dendrobium* orchids have been used as traditional medicine, beauty products, functional diet as well as ingredients in food and drink

(Gutiérrez, 2010). Natural antioxidant agents extracted from soft cane type *Dendrobium* such as flavonoids and phenolic substances exhibited high amount of antioxidant proportion (Wang et al., 2016; Khoo et al., 2017; Muddathir et al., 2017). Since Thailand mainly produces cut *Dendrobium* hybrids and *Dendrobium* "Sonia Jo Daeng" is marketed as one of leading cut flowers exported from Thailand. There is also the demand for edible flowers and their parts claimed not only the sensational feel but also their bioactive functionalities. Therefore, the aim of this research was to analyze the amount of bioactive substances such as total phenolic compounds, anthocyanins, polysaccharides, and antioxidant activity from various parts (leaves, stems, roots, flowers and peduncles) of 1- and 4-years-old *D.* 'Sonia Jo Daeng'. The results can be used as a guideline to increase the value of agricultural products or useful information of phytochemical extracts for further use in cosmetic production and/or health supplementary products.

2. MATERIALS AND METHODS

2.1 Plant materials and extraction methods

Plant materials of *D.* 'Sonia Jo Daeng' were collected from 1- and 4-year-old plants. The whole parts of leaves, stems, roots, flowers and peduncles were collected and cleaned by distilled water, air-dried and then powdered by grinding in liquid nitrogen and kept in a test tube in the refrigerator (-20°C) before used. The extraction and analysis technique was following Macwan et al. (2010) and Sutharut and Sudarat (2012) with some modifications. The aliquote of one gram of tissue samples was transferred into a new 15 ml-test tube, 3 ml methanol were added and vortexed for 30, and the mixture was then incubated at 60°C for 20 min. During the incubation, the sample extract was mixed twice using vortex. High speed centrifuge was used at 10,000 rpm for 10 min to separate plant debris. The supernatant was separated to a 10-ml flask. The pellet was re-extracted with 3 ml of methanol. Then the supernatant was isolated as previously described and combined with the earlier supernatant and was adjusted to 10 ml. The extract was kept in the refrigerator (4°C) until further use.

2.2 Phytochemical analyses

2.2.1 Total phenolics

A series of gallic acid standard solutions were used to measure total phenolic contents of all extractions as reported by Macwan et al. (2010) and Singleton and Rossi (1965) with some modifications. A standard or 0.1 ml of sample was added with 1.5 ml of 7.5% (w/v) Na_2CO_3, vortexed the mixture vigorously for 3 min and then added with 0.5 ml of 10% Folin-Ciocalteu's phenol reagent. The conglomeration was incubated for 30 min at room temperature and the absorbance was measured at 765 nm. Results were expressed as mg of total phenolic content per g of sample powder as gallic acid equivalent (mg GAE/g DW).

2.2.2 Total anthocyanins

The total anthocyanin content (TAC) was determined by the pH-differential method following Lee et al. (2005) and Sutharut and Sudarat (2012) with slightly modification. First, extracted solution (1 ml) was transferred into 10 ml glass vial for preparing two sample dilutions: one was adjusted the volume with potassium chloride buffer (pH 1.0), and another was adjusted the volume with sodium acetate buffer (pH 4.5). Both dilutions were equilibrated for 15 min and the absorbance was measured with UV-VIS spectrophotometer (Optizen 3220UV, Daejeon, South Korea) at 510 and 700 nm, which was compared with distilled water. Cyanidin-3-O-glucoside was used as a standard. All measurements were accomplished between 15 and 60 min after sample establishment. The absorbance of the diluted sample (A) was calculated as follows:

$$A = (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5} \quad (1)$$

the monomeric anthocyanin pigment concentration was calculated using the equation as described by Chaovanalikit (2011):

Monomeric anthocyanin pigment (mg/l)
$= (A \times MW \times DF \times 1000)/ (\varepsilon \times L)$ (2)

where MW is the molecular weight of cyanidin-3-glucoside, 449.2, DF is the dilution factor, and ε is the molar absorptivity, which equal 26,900.

2.2.3 Determination of crude polysaccharides

Crude polysaccharide extraction was completed according to Wang et al. (2018), Luo and Fan (2011) and Albalasmeh et al. (2013). The collected samples were rapidly dried at 60°C. The samples were ground using a mortar and pestle. The 20-ml distilled water was added to one gram of each sample, and the mixture was vortex mixed by ultrasonic cleaner (GT SONIC-D3, China) at 60°C for 20 min. The mixture was filtered by vacuum filter. The residue was re-mixed with 70% ethanol and incubated at room temperature for 12 h. Then, the supernatant in each tube was separated by centrifugation at 6,000 rpm for 10 min. The sediment was collected and washed with ethanol and separated by centrifugation again. The collected 0.05-g sediment was dissolved with 50-ml distilled water. The extracted solution was kept at 4°C until analysis. Extraction of crude polysaccharides was determined by the phenol-sulfuric acid method (Albalasmeh et al., 2013), with glucose solutions (50, 100, 150, and 200 µg/ml) as the standards. The polysaccharide extraction yield (Y) was calculated as follows:

$Y (\%) = (100 \times W_{ps})/W_{sample}$ (3)

where W_{ps} is the weight of the crude polysaccharides and W_{sample} is the weight of the sample. Results of soluble polysaccharides were expressed as mg of glucose per g of crude polysaccharides (mg glucose/g polysaccharide)

2.2.4 Antioxidant activity analysis

Antioxidant activity of the methanol crude extracts from different parts of *D.* 'Sonia Jo Daeng' were analyzed by 1,1-diphenyl-2-picryl-hydrazil (DPPH) free radical scavenging method (Sutharut and Sompong et al., 2011; Sudarat, 2012). The 0.0634 mM DPPH solution was prepared in a methanol solvent. Two milliliters of DPPH solution was mixed with 0, 25, 50, 100 and 150 µl of the orchid crude extracts or 0.5 mM trolox (a standard antioxidant compound). The mixtures were vortexed for 3 s and then the absorbance was rapidly measured at 515 nm using UV-VIS spectrophotometer (Optizen 3220UV, Daejeon, South Korea) (Abs_{t0}). The reaction solution was incubated in a dark condition at room temperature for 30 min. Then the absorbance of the reaction mixture was monitored again to obtain the Abs_{t30} values. All analyses were performed from 4 biological replicates. The percentage of DPPH inhibition was calculated using the following formula:

% DPPH inhibition
$= (Abs_{t0} - Abs_{t30} / Abs_{t0}) \times 100$ (4)

where, Abs_{t0} is the absorbance of the reaction mixture after incubation for 0 min, Abs_{t30} is the absorbance of the reaction mixture after incubation for 30 min.

The percentage of DPPH inhibition was plotted against each quantity of the extract solution. The concentration of the orchid crude extracts that exhibit 50% DPPH radical scavenging activity was defined as IC_{50}. The trolox equivalent antioxidant activity (TEAC) was calculated from the ratio of the slopes of the regression lines of the samples and the trolox solutions.

2.3 Statistical analysis

The data were presented as mean±standard deviation. Statistical analysis was conducted with variance using SPSS software. Treatment mean comparisons were analyzed using Duncan's multiple range test (DMRT) at *p*<0.05. All analyses were performed in four biological replicates.

3. RESULTS AND DISCUSSION

3.1 Total phenolic contents in *D.* 'Sonia Jo Daeng' extracts

The extracts from various parts of *D.* 'Sonia Jo Daeng' were significantly different in total phenolic contents (Figure 1). The extract from leaves of 4-year-old plants had the highest total phenolic content (7.23±0.80 mg GAE/g DW) followed by roots of 4-year-old plants, and flowers of 1- and 4-year-old plants (6.79±1.88, 6.63±0.67 and 6.07±0.62 mg GAE/g Dw, espectively). The extracts from stems, peduncles and roots of 1-year-old plants had the lowest total phenolic contents (1.90±0.13, 1.97±0.35 and 1.81±0.12 mg GAE/g DW, respectively). The results indicated that the older organs, such as leaves and roots significantly increased the amount of phenolic compounds. Similar results were found in coriander harvested at 45, 50, 55 and 60 days after seed sawing had significantly different in total phenolic contents 156.77, 198.00, 195.07, and 212.50 mg GAE/100 g DW, respectively (Chantaraponpan et al., 2018). Since, the phenolic compounds in plants are mainly produced when stress occurred, thus the older plant may encounter inappropriate environment conditions, leading to experience ROS-stress induction, thus the increase level of phenolic through plant-stress respond mechanism (Ali, 2014).

Figure 1 Total phenolic content in mg gallic acid equivalents (GAE) per gram of dry weight from different parts of 1- and 4-year-old *Dendrobium* 'Sonia Jo Daeng'

Note: The same letters indicate that the data are not significantly different (p<0.05) as tested by Duncan's multiple range test

3.2 Total anthocyanin contents

The extracts from various parts of *D.* 'Sonia Jo Daeng' were significantly different in anthocyanin content (Figure 2). The highest anthocyanin content was found in the flowers harvested from 1- and 4-year-old plants with no significantly difference from each other (13.28±2.09 mg/l and 10.21±6.35 mg/l, respectively) (Figure 2). The results of anthocyanin content from 1- and 4-year-old plants were found to be higher in flowers than leaves and peduncles (Figure 2). Anthocyanin in flowers is responsible for attracting the pollinators and help seed distribution in plants (Yamasaki, 1997) but anthocyanin in leaves may function to prevent the damaging of shad-adapted chloroplasts from high light intensity as reported in *Quintinia serrata* A. Cunn.'s leaves (Gould et al., 2000). Anthocyanins are phenolic compound that has potent antioxidant properties (Biswas et al., 2017). The nutraceutical and pharmaceutical effects of anthocyanins and its applications as natural food colorants were reviewed by Khoo et al. (2017). Thus, the extracts from flowers and leaves of *D.* 'Sonia Jo Daeng' would be suitable for using as an ingredient in functional foods.

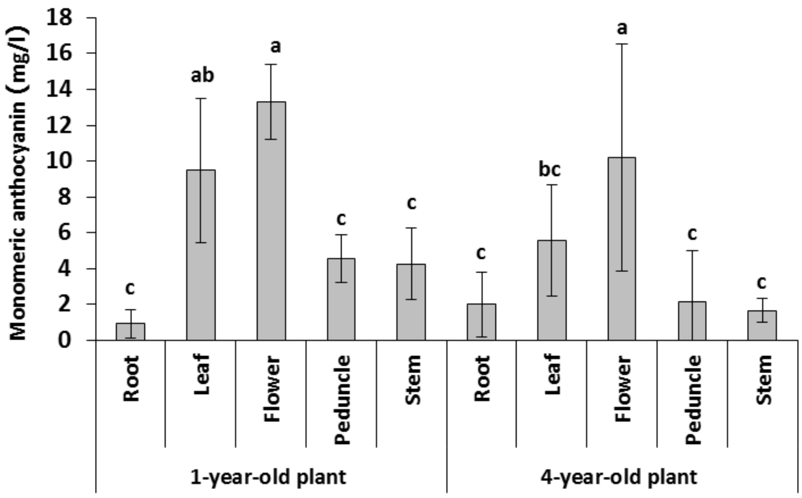

Figure 2 Total amount of anthocyanin in the form of mg per gram of dry weight from different parts of 1- and 4-year-old *Dendrobium* 'Sonia Jo Daeng'

Note: The same letters indicate that the data are not significantly different ($p<0.05$) as tested by Duncan's multiple range test

Anthocyanin studies in other orchids indicated that flowers of *Vanda coerulea* had 6.47 mg per 100 grams of fresh weight anthocyanin content (Junka et al., 2008). The study in *Phalaenopsis* indicated that purple flowers had higher anthocyanin contents when compared to white and yellow flowers (Nguyen et al., 2018). Thus, flower colors and stages were linked to the amount of anthocyanin contents in plants.

3.3 Crude polysaccharides

The extracts from various parts of *D.* 'Sonia Jo Daeng' were significantly different in crude polysaccharides (Figure 3). The percentage of polysaccharide obtained from 1-year-old plants was found in the flowers more than the stems and leaves. Yield of polysaccharides from 4-year-old plants showed that among all tissues analysed, flower parts had the highest percentage content. The lowest polysaccharide yield was found in the peduncles harvested from 1-year-old plants (Figure 3).

Total sugar levels were examined by the phenol-sulfuric acid protocol with glucose as a standard. The total sugar from 1-year-old plants was found to be the highest in flowers as compared with those from leaves and peduncles. The extracts from 4-year-old flowers had the maximum total sugar contents (153.95±12.63 mg glucose/g polysaccharide) (Figure 4). Luo et al. (2009) also studied the crude polysaccharide extraction from psuedobulb of *D. nobile* Lindl. and reported that polysaccharide from soft cane *Dendrobium* also showed the antioxidant activity, thus future experiment will conduct on polysaccharide extracted from *D.* 'Sonia Jo Daeng'.

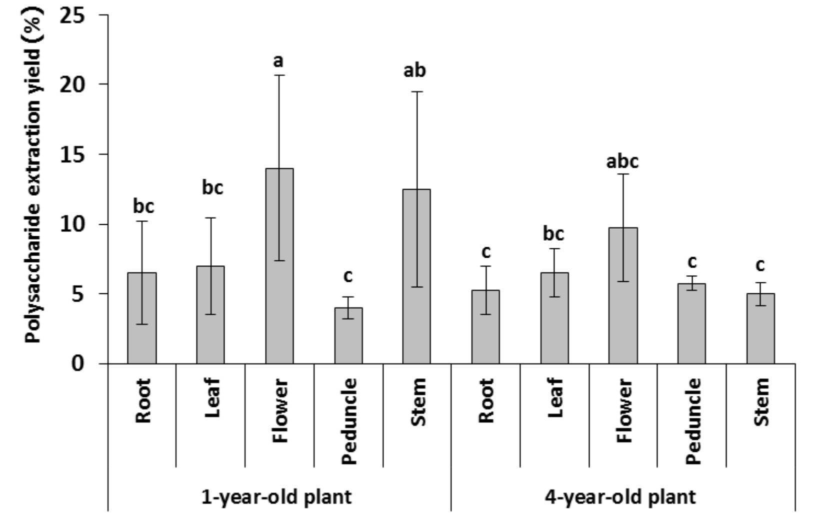

Figure 3 Polysaccharide extraction yield (%) from different parts of 1- and 4-year-old *Dendrobium* 'Sonia Jo Daeng'

Note: The same letters indicate that the data are not significantly different ($p<0.05$) as tested by Duncan's multiple range test

Figure 4 Total sugars in the form of mg glucose/ g polysaccharide from different parts of 1- and 4- year-old *Dendrobium* 'Sonia Jo Daeng'

Note: The same letters indicate that the data are not significantly different ($p<0.05$) as tested by Duncan's multiple range test

3.4 Antioxidant activity

The extracts from various parts of *D.* 'Sonia Jo Daeng' were significantly different in antioxidant activity (Figures 5 and 6). The IC_{50} was inversed with antioxidant activity. The highest IC_{50} was found in the peduncles of 1-year-old plants (0.0073±0.001 mg/ml). The lowest IC_{50} was found in the flowers and roots of 1-year-old plants (0.0018±0.001 and 0.0018±0.001 mg/ml, respectively) (Figure 5). In 1-year-old *D.* 'Sonia Jo Daeng', the TEAC indicated that the extracts from flowers had higher antioxidant activity than those of stems, leaves, roots and peduncles. However, the antioxidant activity was found maximum in roots of 4-year-old *D.* 'Sonia Jo Daeng' (Figure 6).

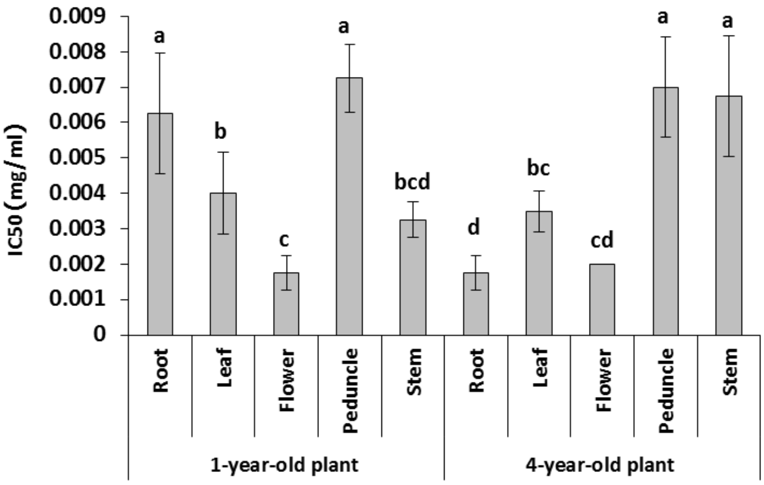

Figure 5 Antioxidant activities (IC_{50}) from different parts of 1- and 4-year-old *Dendrobium* 'Sonia Jo Daeng'

Note: The same letters indicate that the data are not significantly different ($p<0.05$) as tested by Duncan's multiple range test

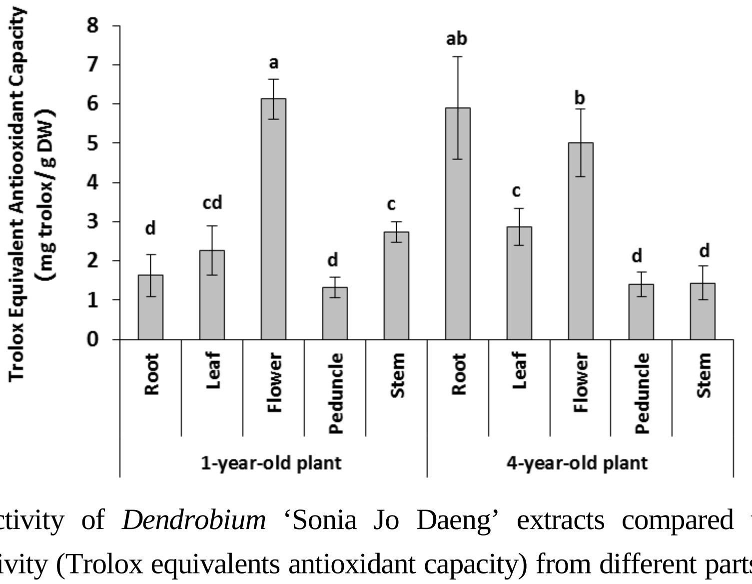

Figure 6 Antioxidant activity of *Dendrobium* 'Sonia Jo Daeng' extracts compared with standard trolox antioxidant activity (Trolox equivalents antioxidant capacity) from different parts of 1- and 4-year-old *D.* 'Sonia Jo Daeng'

Note: The same letters indicate that the data are not significantly different ($p<0.05$) as tested by Duncan's multiple range test

The results suggested that antioxidant activity was significantly higher in roots of the older plant. Moreover, the results of antioxidant activity were correlated with the amount of total phenolic compounds in which the maximum phenolic content and antioxidant activity was found in flowers of 1- and 4-year-old plants and in roots of 4-year-old plants. Similar results were found in the extraction from rhizome of Indian medicinal plant, *Polygonatum verticillatum* (L.); it indicated that antioxidant activity from the extraction was also associated with the phenolic contents (Kumar and Patra, 2018). The experiment conducted on different cultivars of sweet potato revealed that DPPH radical-scavenging activity was also correlated to the phenolic level (Oki et al., 2002). Phenolic compounds known as bioactive compounds that have various biological activities to alleviate several chronic diseases, and they were commonly found in the plant kingdom especially in herbs, spices, fruit peels and vegetables (Kähkönen et al., 1999). It has been reported that the pigments or colors of the petals correlated to the phenolic contents (Lu et al., 2019). However, petal color does not always show the stronger antioxidant activities in the dark color flowers since the extract from the white flower color of *D.* 'Shavin White' had higher antioxidant activities than *D.* 'Sonia Pink', *D.* 'Snow Rabbit' and *D.* 'Sonia', respectively (Athipornchai and Jullapo, 2018). Our results suggested that different plant parts of *D.* 'Sonia Jo Daeng' contained variable amount of bioactive compounds. Since *D.* 'Sonia Jo Daeng' has been propagated in a large area with massive flower production throughout the year, at some periods of the year, the productions are over supply. By offering this information, it could be possible for the new market of obtianing *Dendrobium* bioactive compounds that are suitable for dietary and medicinal application. This may solve the problem of oversupply of this orchid type and help the farmers to increase their additional income.

4. CONCLUSION

The highest total phenolic compounds were found in the flowers of the 1-year old *D.* 'Sonia Jo Daeng' and 4-year old roots, leaves and flowers. The highest anthocyanin content was found in the flowers of *D.* 'Sonia Jo Daeng' at both 1 year old and 4-year old. The highest amount of polysaccharide was found in flowers and stems of *D.* 'Sonia Jo Daeng' of the 1 year old and flowers of *D.* 'Sonia Jo Daeng' of the 4 years old.

The highest antioxidant activity was found in the flowers of *D.* 'Sonia Jo Daeng' of the 1-year old and roots of the 4-year old. Levels of antioxidant were corelated to the amount of total phenolic compounds, anthocyanin content and polysaccharides. However, the activity from the 4-year old roots was only corelated to the amount of phenolic compounds.

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