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# Tyrosinase inhibitory and antioxidant activities of Orchid (*Dendrobium* spp.)

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ABSTRACT

Ethanol extracts of the flowers from four *Dendrobium* species cultivated in Thailand including Sonia, Sonia Pink, Snow Rabbit and Shavin White were prepared using the maceration method at room temperature and their total phenolic and total flavonoid contents together with antioxidant activities using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and phosphomolybdenum assay were studied. The Shavin White extract (5.34 ± 0.30 mgGAE $g^{-1}$) showed the highest total phenolic content followed by those of Sonia and Sonia Pink, respectively whereas the Sonia Pink extract (4.01 ± 0.12 mgGAE $g^{-1}$) showed the highest total flavonoid content. The Shavin White extract also showed stronger antioxidant activities than Sonia Pink, Snow Rabbit and Sonia, respectively. All ethanolic extracts were evaluated for tyrosinase inhibitory activity using the modified dopachrome assay with L-tyrosine and L-DOPA as substrates. It was found that all extracts showed the strongest inhibition of tyrosinase in the first step (L-tyrosine) of melanin synthesis whereas they showed lower inhibitory activity in the second step (L-DOPA) of melanin synthesis. Consequently, the flowers of *Dendrobium* species may have dietary and medicinal applications.



## 1. Introduction

Health and beauty are currently topics of great interest for the general public. Several diseases may be caused by reactive oxygen species (ROS) generated by normal metabolic processes resulting in oxidative damage to human cells. Herbs have played an important role in drug discovery and were the basis of most early medicines. Various chemically active compounds from herbs are present in the herbs amongst which phenolic compounds have received much attention mainly due to their multifaceted role in the treatment of various degenerative diseases and aging related disorders. Therefore, herb-based products including dietary supplements, pharmaceuticals and foods are believed to be useful in protecting humans against oxidative damage and diseases such as cancer, inflammatory, cardiovascular and degenerative diseases with little side effects to the body (Halliwell and Gutteridge 1984; Butler 2005; Jo et al. 2012; Huang et al. 2005; Zengin et al. 2015). Thailand has various types of medicinal plants for the treatment of various chronic diseases. The largest group of flowering plants in the world is the Orchidaceae family distributed mainly in the tropical regions. Several classes of phytoconstituents isolated from therapeutically used orchids have been studied for their biological activities, especially in the fields of cancer, inflammation, and neurodegeneration (Sut et al., 2017). Moreover, these orchid plants have been used in cosmetic, dietary supplement and food (Rosa 2010). Some orchid extracts showed inhibition of tyrosinase which is a copper-containing enzyme also known as polyphenol oxidase (PPO). Tyrosinase, widely found in plant and mammalian cells, regulates melanogenesis within melanocytes. Melanogenesis is a physiological process in the synthesis of melanin pigments, which are essential for protecting human skin against radiation. This process can be controlled by inhibiting the activity of tyrosinase. Tyrosinase inhibitors are topically used for treating localized hyperpigmentation in humans. Natural antioxidant agents such as phenolic compounds and flavonoids showed high antioxidant capacities and are good inhibitors of tyrosinase activity and melanin production (Wang et al. 2016; Biswas et al. 2017; Muddathir et al. 2017).

*Dendrobium* species (number over 1100) of the Orchidaceae family, cultivated in Thailand, have been credited as a traditional medicine over the centuries in Asia, Europe and Australasia. *Dendrobium* species such as *D. catenatum*, *D. crepidatum*, *D. loddigesii*, *D. nobile* and *D. officinale* have been used in cosmetic, traditional medicine and food to treat many diseases and ailments caused by oxidative stress in the body (Rosa 2010; Ng et al. 2012; Bhattacharyya et al. 2016). Moreover, *Dendrobium* species are marketed as cut flowers plants and these orchid species are a major stake holder in the worldwide cut flower market because they are outstanding in possessing diverse shapes, forms and colors (Bhattacharyya et al. 2014). However, marketing of these orchids often involves the rejection of substantial quantities which necessitates

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proper disposal. Therefore, the aim of this study was to evaluate the chemical composition including the total phenolic and total flavonoid contents and antioxidant activities using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and phosphomolybdenum assay of ethanolic extracts from the flowers of *Dendrobium* species including Sonia, Sonia Pink, Snow Rabbit and Shavin White. Moreover, the tyrosinase inhibitory activities of *Dendrobium* ethanolic extracts were also measured to see if these extracts can be used as ingredients of cosmetics and pharmaceutical agents so that the amount of orchid wastes can be reduced.

## 2. Material and methods

### 2.1. Materials

The flowers of *Dendrobium* species including Sonia, Sonia Pink, Snow Rabbit and Shavin White were collected in March 2013 at Burana Orchid Farm, Nakhon Pathom Province, Thailand. Dimethyl sulfoxide (DMSO), Folin–Ciocalteu reagents, aluminum trichloride ($AlCl_3$), DPPH, ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}.4H_2O$), lyophilized mushroom tyrosinase (E.C.1.14.18.1), L-tyrosine, L-DOPA (3,4-dihydroxyphenylalanine), gallic acid, quercetin, ascorbic acid, kojic acid were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). All the organic solvents and chemicals used in this study were of analytical grade.

### 2.2. Preparation of ethanolic extracts from the flowers of Dendrobium species

Fresh flowers (2.0 kg each) of Sonia, Sonia Pink, Snow Rabbit and Shavin White were cut into small pieces and macerated with 2.0 L of 95% ethanol at ambient temperature for 2 weeks and the extracts were filtered. The solvent was then removed under reduced pressure at 50 °C to give the crude extracts which were freeze-dried and then refrigerated until further use.

### 2.3. Total phenolic contents

The total phenolic contents of the respective ethanolic extracts from the flowers of *Dendrobium* species were determined by a spectrophotometer (Specord-210 Plus UV/VIS), using Folin–Ciocalteu's phenol reagent (Majhenic et al. 2007). Each dry extract was dissolved in methanol (5.00 mg/mL) and 0.3 mL of the extract was reacted with 0.7 mL of 10% Folin–Ciocalteu's phenol reagent. The reaction was pre-incubated at room temperature for 5 min. After 5 min, 2.5% $Na_2CO_3$ solution (1.0 mL) was added and the reaction mixture was kept for 30 min in the dark. The absorbance of the reaction mixture was measured at 760 nm. Quantification of the total phenolic contents was based on a standard curve generated with gallic acid at 760 nm using the following equation: $Abs = 0.0977x + 0.1054$ ($R^2 = 0.9989$), where Abs is the absorbance and $x$ is the concentration (μg/mL). All tests were conducted in triplicate and averaged. The results were expressed as mg of total phenolic contents per 1.0 g of dry extract as gallic acid equivalents (GAE).

### 2.4. Total flavonoid contents

The aluminum chloride colorimetric method was used to estimate the total flavonoid contents in the extracts (Arvouet-Grand et al. 1994). The dry extracts were dissolved in methanol (5.00 mg/ml) and 0.3 ml of the extract was mixed with 1.7 ml of 2% aluminum chloride ($AlCl_3$) methanolic solution. The absorbance of the reaction mixture was measured at 415 nm by a spectrophotometer (Specord-210 Plus UV/VIS), after incubation 10 min at room temperature. The total flavonoid contents in the extracts were determined using a standard curve established with quercetin ($y = 0.0507x + 0.0243$, $R^2 = 0.9995$), and the results expressed as mg of total flavonoid contents per 1.0 g of dry extract as quercetin equivalents (QE). All tests were conducted in triplicate and averaged.

### 2.5. DPPH radical scavenging activity

The electron donation or hydrogen atom ability of the extracts was measured from the bleaching of a purple colored methanol solution of DPPH (Braca et al. 2002). A stock solution of each extract (5.0 mg/ml) was prepared in methanol. The extracts (0.2 mL) were added to 1.8 mL of freshly prepared DPPH methanol solution (0.05 mM) and mixed. Decreases of the absorbance of the tested samples were monitored at 517 nm using a UV-Vis spectrophotometer (Specord-210 Plus UV/VIS). Inhibition of free radical DPPH in percent (%) was calculated as follows, % Inhibition = [(Ablank–Asample)/Ablank] × 100, where Ablank is the absorbance of the control reaction (containing all reagents except the test compound), and Asample is the absorbance of the test compound. The sample concentration providing 50% inhibition ($IC_{50}$) was calculated by plotting the inhibition percentages against the concentrations of the sample. All tests were carried out in triplicate and $IC_{50}$ values were reported as means. Gallic acid, ascorbic acid and quercetin as the reference antioxidant reagents were prepared in methanol.

### 2.6. Phosphomolybdenum antioxidant assay

The total antioxidant activity of the extracts was evaluated by a spectrophotometer (Specord-210 Plus UV/VIS), using phosphomolybdenum reagent (Prieto et al. 1999) which was based on the reduction of Mo(VI) to Mo(V) by the extract with subsequent formation of a green phosphate-Mo(V) complex under acidic condition. A stock solution of each extract (5.0 mg/ml) was prepared in methanol and 0.2 mL of the extract was mixed with 1.8 mL of phosphomolybdenum reagent (600 mM sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and the reaction was incubated at 78 °C for 30 min. Then the absorbance of reaction mixture was measured at 695 nm. The total antioxidant activity of the extract was determined using a standard curve established with ascorbic acid ($y = 0.0309x + 0.0093$, $R^2 = 0.9991$), and the results expressed as mg of total antioxidant activity per 1.0 g of dry extract as ascorbic acid equivalents (AAE). All tests were conducted in triplicate and averaged.

### 2.7. Tyrosinase inhibitory activity

The mushroom tyrosinase activity of the extracts was measured by using spectrophotometric method with L-tyrosine and L-DOPA as substrates and kojic acid as a standard tyrosinase inhibitor (Masuda et al. 2005). The extract were first dissolved in methanol at a concentration of 20.0 mg/mL, and then diluted to different concentrations using methanol. Subsequently, a 120 μL of 50 mM sodium phosphate buffer pH 6.8 was mixed with 20 μL of the extract solution and 20 μL of tyrosinase (800 unit/mL in 50 mM phosphate buffer, pH 6.8) and transferred to a 96-well plate and the reaction mixture was pre-incubated at room temperature for 10 min. Finally, 40 μL of 3.0 mM L-tyrosine or L-DOPA substrate was added to the well plate and incubated for 10 min. The absorbance of the reaction mixture was measured at 492 nm using EPOCH-2 microplate reader (Biotek). The inhibition of tyrosinase activity in percent (%) was calculated as follows, % Inhibition = [(Ablank–Asample)/Ablank] × 100, where Ablank is the absorbance of the control reaction (containing all reagents except the test compound), and Asample is the absorbance of the test compound. The sample concentration providing 50% inhibition ($IC_{50}$) was calculated by plotting the inhibition percentages against the concentrations of the sample. All tests were carried out in triplicate and $IC_{50}$ values were reported as means.