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Biological activity and phytochemical profiles of *Dendrobium*: A new source for specialty cosmetic materials

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ABSTRACT

Dendrobium is the major tropical orchid cut flower of the global market. This industrial floriculture has been documented as herbal remedies. However, only 40% of the crop is qualitied for commercial. Preparation of the orchid as a specialty cosmetic ingredient is presented. Maceration of the flower in 70% EtOH or water gave feasible yields of extracts. Technical data in terms of total anthocyanin, phenolic and tannin contents, astringency and *in vitro* antioxidant activities by means of ABTS, DPPH and FRAP assays were reported as per enzyme inhibitory effects against aging and hyperpigmentation. Pelargonidin and sinapic and ferulic acids were determined as the major actives. Safety profiles of the 70% EtOH extract was wider than the water fraction as examined in human fibroblasts and B16F10 melanoma. The extracts exhibited antioxidant and pro- and active-form of MMP-2 inhibitory effects in fibroblasts. In addition, their anti-melanogenesis activities in melanoma were proved by tyrosinase and TRP-2 suppressions. The physiochemical profiles and stability of the orchid flower extracts were included. Orchid flower is therefore highlighted as a safe and efficient ingredient potential for skin aging and lightening treatment. Multiple use of orchid flower as the specialty industrial material is encouraged with the information feasible for the industrial practice.

1. Introduction

Orchid is one of the important industrial floriculture with global revenue of more than 400 million US dollars. Thailand is the global number 1 producer and exporter of tropical orchid with 8606 t exportation during January–February of 2017 that costs 12.51 million US dollars. This revenue is 9.64% increasing from the same period of the year 2016 of which *Dendrobium* Sonia Earsakul is the highest exported orchid (94.7% to total orchid cut flower) due to its attractive anthocyanin red-purple color. However, only 40% of the total produced orchid is qualified to be exported. The rest of the floriculture is therefore domestically consumed with some portion that treated as waste (Thailand Ministry of Commerce, 2017).

Dendrobium has been documented in Chinese Pharmacopeia as a folk medicine due to its phytochemical actives i.e. phenolics, anthocyanins and polysaccharides (Hossain, 2011; Ng et al., 2012). Thus, low quality of *Dendrobium* cut flower insufficiently traded and discarded from the orchid production is worthy to be explored for specialty industrial materials application (Hossain, 2011) including for cosmetics. Cosmetic products of natural sources especially those of sustainable and ecological friendly are highly impact (Lubbe and Verpoote, 2011) on the consumer preferences and they are conceivable more safety (Kanlayavattanakul and Lourith, 2015; Lourith and Kanlayavattanakul, 2017). However, research and technical data supports an application of *Dendrobium* for cosmetics are sparely to be reported.

The present study is objected to reveal the potential of *Dendrobium* as a multiple crop uses for cosmetic industry. Preparation of the orchid waste as a standardized flower extract for skin aging prevention and treatment products is presented herein. Quality control in terms of total anthocyanin and phenolic contents were reported including total tannin content and astringency. In addition, antioxidant activities, enzyme inhibitory effects and safety and cellular activities in cell cultures of fibroblasts and melanomas were undertaken with analysis of anthocyanins and phenlolics. The extract stability profile was enclosed herein. The presenting results are therefore ensuring the application of orchid flower extract as a phytochemical source of specialty ingredient for anti-aging and skin lightening products.

2. Materials and methods

All of the reagents and chemicals used for extractions were of commercial grade. Those for *in vitro* and cell culture assays were of

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analytical grade unless otherwise specified. Standards and solvents for UPLC analysis were of HPLC grade.

2.1. Preparation of orchid flower extracts

Fresh *Dendrobium* Sonia Earsakul flowers cultivated in Nakhon Pathom, the largest floriculture province of Thailand, were washed with tap water, dried (40 °C) and grinded (< 2 mm). The extract preparation was by maceration in either 95% EtOH or 70% EtOH or 50% EtOH or water for 15, 30, 60, 180, 360 and 1440 min, separately, with shaking at 150 rpm. The extract was concentrated to dryness under *vaccuo*. The extractive yield was calculated, and each of the extraction condition was repeated for more 2 times.

2.2. Quality control and standardization of the orchid flower extracts

2.2.1. Total anthocyanin content (TAC)

TAC was assessed by means of a pH differential method modified from Wrolstad (1976) in KCl (0.2 M, pH 1.0) and $C_2H_3O_2Na$ (1 M, pH 4.5). The sample in each buffer was absorbency recorded at 520 and 700 nm using a microplate reader (ASYS, Biochrome UVM340, UK). TAC was calculated with the extinction coefficient for cyanidin-3-glucoside as followings;

$$\text{Absorbance} = (A_{520nm} - A_{700nm})pH1.0 - (A_{520nm} - A_{700nm})pH4.5$$

and expressed as mg cyanidin equivalent per 100 g extract (mg Cyn/100 g extract). The assay was undertaken in triplicate.

2.2.2. Total phenolic content (TPC)

The extracts were additionally assessed for their biologically active contents by means of TPC in a comparison with gallic acid using tFolin-Ciocalteu reagent. Samples with a serial concentration were mixed with Folin-Ciocalteu reagent, followed by an addition of 7.5% Na_2CO_3 and water. The reaction mixtures were incubated for 1 h under the ambient temperature. Absorbencies at 750 nm were determined by the microplate reader. The results were reported in g gallic acid equivalent/100 g extract (g GAE/100 g extract). The assessment was repeated for more 2 times (Kanlayavattanakul et al., 2017).

2.2.3. Total tannin content (TTC) and astringency

The orchid flower extract dissolved in a mixture of DMSO and water, was mixed with 5% Na_2CO_3 and 1 N Folin-Ciocalteu reagent. The reaction mixture was incubated for 60 min, followed by an absorbance recorded at 725 nm. Of which, tannic acid was used as the standard. TTC was expressed in μg tannic acid equivalent/g extract (μg TAE/g extract) Astringent activity was measured by mixing the sample in EtOH with hemoglobin in phosphate buffer saline (pH 6.8) at 1:1 ratio. The whole was centrifuged at 3000 rpm for 10 min, the supernatant was analyzed at 407 nm in a comparison with tannic acid, the positive control, and the results were reported in mg tannic acid equivalent/g extract (mg TAE/g extract). All of the assays were undertaken in triplicate (Lourith and Kanlayavattanakul, 2017).

2.3. In vitro radical scavenging activities

2.3.1. $ABTS^{\cdot+}$ scavenging activity

Absorbance for ABTS containing $K_2S_2O_8$ and EtOH mixed with samples was recorded following 5 min incubation at 750 nm. The antioxidant efficiency (IC_{50}) was compared to that of standard ascorbic acid in different concentrations that generating calibration curves. Measurements were performed in triplicate (Kanlayavattanakul et al., 2015).

2.3.2. DPPH· scavenging activity

Antioxidant activity, on the basis of a DPPH assay, was assessed in parallel. DPPH in absolute EtOH was allowed to react with ascorbic acid to generate a calibration curve. The scavenging activity of the sample against DPPH· was monitored at 517 nm. The radical terminating capability (IC_{50}) was calculated in comparison with the standard values. The experiments were performed in triplicate (Kanlayavattanakul et al., 2015).

2.3.3. Ferric reducing ability of plasma (FRAP)

Ferric reducing ability of plasma (FRAP) of the extract was examined. A FRAP reagent was prepared in a 2,4,6-tri(2-pyridyl)-S-triazine (TPTZ) solution with HCl, $FeCl_3$, and acetate buffer. The extract was reacted with the FRAP reagent, and absorbance recorded at 595 nm. The reducing power was determined in triplicate and expressed as an equivalent concentration (EC) to that of $FeSO_4$ (Kanlayavattanakul et al., 2017).

2.4. In vitro enzyme inhibitory activities

2.4.1. Collagenase inhibition

Anti-collagenase assay was undertaken by mixing tricine buffer (60 mM, pH = 7.5) with collagenase (ChC − E.C. 3.4.23.3) at a concentration of 0.8 units/mL and 2 mM of *N*-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala (FALGPA). The absorbance was recorded at 340 nm before and after an incubation of the test sample with enzyme and FALGPA. The anti-collagenase activity was compared with epigallocatechin gallate or EGCG (Lourith et al., 2017). The assay was performed in triplicate.

2.4.2. Elastase inhibition

Elastase inhibitory activity was examined. The reaction mixture containing Tris-HCl buffer (0.2 mM, pH = 8.0), porcine pancreatic elastase (PE – E.C. 3.4.21.36) with a concentration of 3.33 mg/mL and *N*-succinyl-Ala-Ala-Ala-*p*-nitroaniline (AAAPVN) (1.6 mM) was prepared. The absorbance of the mixture was monitored at 410 nm throughout the reaction, and after incubating the test sample for 20 min. The enzyme inhibitory effect was compared with that of ursolic acid (Lourith et al., 2017). The protocol was repeated three times for each sample.

2.4.3. Tyrosinase inhibition

The tyrosinase inhibitory activity was determined using the dopachrome method with *L*-Dopa as a substrate. A sample that was a mixture of a phosphate buffer and mushroom tyrosinase was incubated at 25 °C for 10 min *L*-Dopa was then added to the mixture and incubated (20 min). The absorbance was measured at 490 nm, using a serial dilution of kojic acid as a positive control. The enzyme deactivation efficacy of each extract was monitored. The assay was performed in triplicate, and the inhibitory effect (%) was calculated in a comparison with kojic acid (Kanlayavattanakul et al., 2016).

2.5. Activities in human fibroblasts

Human skin fibroblasts (ATCC® CRL 2097, USA) at 6–13th passage were cultured in 75 cm^2 flask in Dulbecco's Modified Eagle's Medium medium supplemented with 10% Fetal Bovine Serum and 1% penicillin/streptomycin at 37 °C under 5% CO_2. Cells were grown and harvested by 0.25%, w/v trypsin and 0.06 mM EDTA in phosphate buffer saline.

The sulforhodamine B (SRB) assay was used for cell cytotoxicity determination. Cells (1×10^4 cells/well) in 96-well plate were incubated for 24 h, and treated with different concentrations of the samples for 72 h. The adherent cells were fixed, washed and dyed prior to the absorbance measurement at 540 nm. The cell viability was compared with the control treated with absolute EtOH (Chaikul et al.,