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# Potential alternative natural colourant from *Dendrobium* Sonia 'Earsakul'

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**Abstract**

This study aimed to investigate optical properties, colour-changing characteristics, and storage stability of crude aqueous extract of fresh *Dendrobium* Sonia 'Earsakul' (DSE) orchid flower obtained through microwave-assisted extraction. IR spectra of the extract at various pH indicated that anthocyanins were major pigments present in the crude extract. As the pH of the extract increased from 2 to 11, its colour turned from reddish-orange to magenta to purple to blue to green. Crude extract of DSE could be prepared in advance and used within 2 weeks after preparation (if kept at 4°C) without significant reduction of anthocyanin levels ($P<0.05$). The findings showed that DSE had the potential to be utilized as a natural food colourant as an alternative for those from plants containing cyanidin as the major anthocyanin, due to their similarities in colour-changing patterns and high storage stability.

## 1. Introduction

Regional plants or their extracts have been locally utilized as natural colourants for food and non-food applications, especially in small-scale productions of foods and beverages, textiles, and cosmetics. The recent shift in consumers' preference toward the use of natural ingredients has renewed and increased the demand for natural colourants. Crude colour extracts can be obtained from various parts of plants, e.g., flower, leaf, fruit peel or skin, tree bark, or tree root. In the Southeast Asia region, many flowers and other parts of agricultural produce of low economic values are often utilized, e.g., butterfly pea (*Clitoria ternatea*) flower, turmeric (*Curcuma longa*) root, and aril of gac (*Momordica cochinchinensis*) fruit. At household- or small-scale production level, the typical extraction method of natural pigments from regional plants is solvent extraction, using hot water, or acidified or alkaline hot water as solvent. The results are crude colour extracts consisting of natural pigments and other soluble compounds which will later be used 'as-is' (Hafizah *et al.*, 2010; Ahmadiani *et al.*, 2014; Saxena and Raja, 2014; de Boer *et al.*, 2019).

Since the year 2020, the cut orchid flower import-export industry is severely affected by the COVID-19 pandemic mainly due to the collapse of global tourism. The demand for cut flowers for decorations or social events sharply dropped, and the post-pandemic recovery is projected to be slow (Thanthong-Knight, 2020). In the long term, finding new or alternative applications for cut orchid flowers will improve the industry's value and sustainability. Dendrobium hybrids are the most popular tropical orchids for cut orchid import-export business. One of the most well-known Dendrobium hybrids, *Dendrobium* Sonia 'Earsakul' orchid has a deep purple colour, with multiple flowers arranged along one unbranched flowering stem. It is grown, almost exclusively, for exported cut-flower, or used locally in religious events or as food garnish (Chandra *et al.*, 2015). Recently, it is also utilized as an edible flower. Dendrobium orchids are reported to have a high amount of anthocyanins, especially, cyanidin glycoside and peonidin glycoside, a derivative of cyanidin, with peonidin glycoside contributing up to 11%, depending on Dendrobium species and type of hybrids (Kuehnle *et al.*, 1997; Hafizah *et al.*, 2010). The objective of this study was to assess colour characteristics and storage stability of crude extract of fresh *Dendrobium* Sonia 'Earsakul' orchid flower for its alternative applications as a natural colourant or pH indicator.

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## 2. Materials and methods

In this work, fresh *Dendrobium* Sonia 'Earsakul' (DSE) orchid flower (growth stage 5) (Pitakdantham *et al.*, 2011) was purchased from local markets in Bangkok, Thailand, and stored at 4±1°C until used (within 3 days of purchase). Crude extract from DSE was prepared using microwave-assisted extraction (MAE) following the procedure outlined by Netramai *et al.* (2020). MAE condition used was fresh flower to water ratio of 1:3 g/mL, microwave power of 800 W, and extraction time of 8 mins.

Total monomeric anthocyanin pigment content (TAC) in the crude colour extract from DSE was determined using the pH differential method (Lee, 2005). The pH of the DSE extracts were then adjusted to predetermined values between 2.0 and 11.0 (with an increment of 1.0) using colourless buffers (Reagecon, Munster, Ireland). The extracts' pH values were verified using a handheld digital pH meter (PH-200 HM Digital Handheld pH Meter, HM Digital, Inc., Redondo Beach, CA, USA) before further testing (all tests were performed within 1 hr of pH adjustment). The extracts were 1) photographed (Olympus PEN E-PL8, with Olympus M.Zuiko Digital 25mm f18 lens, Olympus (Thailand) Co., Ltd., Bangkok, Thailand); 2) determined their visible spectra (400-700 nm), using UV-Vis spectrophotometer (Shimadzu UV-1280 UV/Vis Spectrophotometer, Bara Scientific Co., Ltd., Thailand); and 3) freeze-dried and then characterized their IR spectra (4,000-750 $cm^{-1}$), using Fourier-transform infrared (FT-IR) spectrophotometer (IRPrestige-21, Shimadzu Corporation, Kyoto, Japan), with single reflection attenuated total reflectance (ATR) – Ge crystal.

To assess the storage stability of the filtered crude extract, the extract was kept in an air-tight glass container, covered with aluminium foil, at 4±1°C, and was sampled periodically to monitor its TAC content until the remaining anthocyanin level was lower than 75% of the initial content. All experiments were performed in triplicate. Data obtained were statistically analyzed using Minitab 19 (Minitab, LLC, PA, USA) at the confidence level of 95% ($\alpha$ = 0.05) with Tukey's adjustment for comparison of the means.

## 3. Results and discussion

Crude colour extract of DSE had a pH of 6.41±0.25 and anthocyanin content of 47.48±2.47 mg cyanidin-3-glucoside equivalents per L of crude extract. Figure 1 shows the colours of DSE extract at pH 2 to 11 and their respective visible spectra. The pattern of the colour change of DSE extract was similar to those of aqueous extract from red cabbage (*Brassica oleraces*) and purple sweet potato (*Ipomoea batatas*), as well as that of *Dendrobium* Sonia 'Red Bom', another Dendrobium hybrid, commercially available as a cut flower (Hafizah *et al.*, 2010). All three plants reportedly have cyanidin-type anthocyanins as major pigments (Hafizah *et al.*, 2010; Ahmadiani *et al.*, 2014; Khoo *et al.*, 2017; Wahyuningsih *et al.*, 2017). Colour characteristics of anthocyanins are influenced by their structural configurations and transformations, and the pH of the surrounding. The presence of anthocyanins in the obtained crude extract was confirmed through the IR spectra of the extract (Figure 2). The extracts displayed the following absorption regions or peaks; 3400 to 3100 $cm^{-1}$ (O-H), 2920 $cm^{-1}$ (C-H, methyl group) and 2850 cm -1 (C-H, methylene group), 1665 and 1554 $cm^{-1}$ (C=C, aromatic), and 1284 and 1079 $cm^{-1}$ (C-O, pyran or flavonoids). The finding was in agreement with previous works on IR spectra of anthocyanins (Wahyuningsih *et al.*, 2017; Favaro *et al.*, 2018).

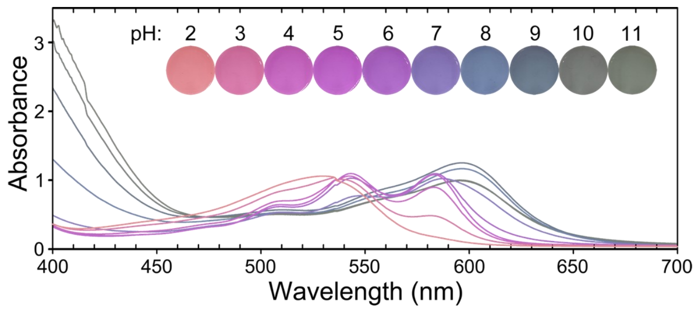


Figure 1. Colours of *Dendrobium* Sonia 'Earsakul' orchid flower crude aqueous extract at pH 2.0 to 11.0 and their corresponding visible absorption spectra.

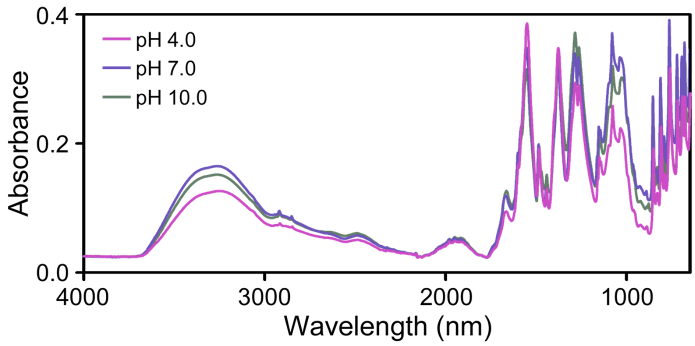


Figure 2. IR spectra of crude *Dendrobium* Sonia 'Earsakul' orchid flower aqueous extract at pH 4.0, 7.0, and 10.0

As the pH of the extract increased from 2 to 11, crude extract visually changed colour from reddish-orange to magenta to purple to blue to green (Figure 1). As the pH of the extract increased, the bathochromic shift of $\lambda_{max}$ was observed, i.e., $\lambda_{max}$ gradually shifted to a higher wavelength. At pH ~2, crude extract of DSE had $\lambda_{max}$ of around 529 nm. Reportedly, at pH below 4, the majority of anthocyanins are in their flavylium cation forms which are reddish-pink. In the pH range of 4 to 6, crude extracts' spectra had 2 distinct peaks, with $\lambda_{max}$ of around 543 and 583 nm. In this pH range, flavylium forms gradually transform to either carbinol pseudobase (colourless) or an unstable form of quinonoidal (bluish colour). This caused the decrease of red colour and turned the extracts' colour to pink and later purple. At alkaline pH, anionic quinonoidal forms are more stable and the extracts' colour turned bluish-green; $\lambda_{max}$ of the extracts moved to ~600 nm or higher. At a very strongly alkaline pH (pH 10-11), crude extracts turned yellowish-green, possibly due to the degradation of anthocyanins (Favaro *et al.*, 2018; Tang *et al.*, 2019).

DSE extract was stored at 4°C and monitored the levels of TAC content (Figure 3). Anthocyanin level of the extract showed no significant decrease for 2 weeks and dropped to less than 75% of initial levels after 6 weeks of storage. Comparatively, previous work done by Hafizah *et al.* (2010) reported a 29% decrease in colour intensity of aqueous extract (pH 7.0) from *Dendrobium* Sonia 'Red Bom' after 72 d of storage at 4°C. On the other hand, Ahmadiani *et al.* (2014) found that anthocyanin contents of red cabbage extracts (in buffer pH 7.0) decreased by 19.1±3.8 to 50.1±5.5% after refrigeration storage of 6 h, depending on red cabbage varieties. This could be because the majority of anthocyanins found in red cabbage is cyanidin glycoside, which is particularly heat-sensitive, and unstable at pH close to neutral (Khoo *et al.*, 2017). For DSE extract, copigmentation between cyanidin- and peonidin-based anthocyanins, as well as other flavonoids might attribute to a more stable anthocyanin level during storage. Additionally, peonidin is more stable at high pH than cyanidin (Kuehnle *et al.*, 1997; Wahyuningsih *et al.*, 2017).

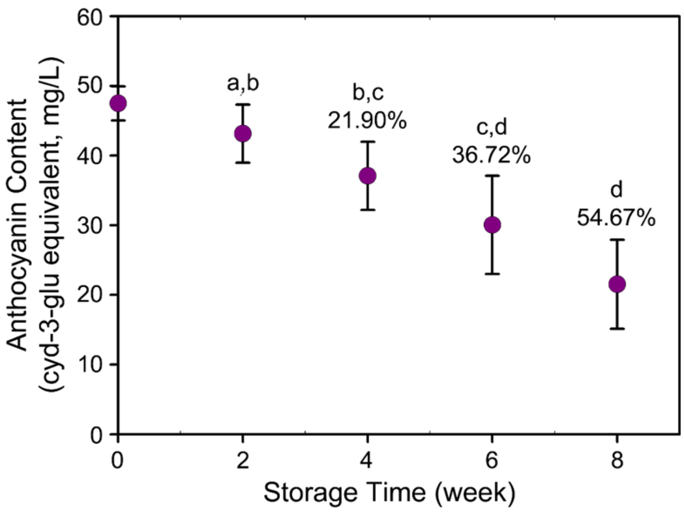


Figure 3. Total monomeric anthocyanin content of *Dendrobium* Sonia 'Earsakul' orchid flower crude aqueous extracts stored at 4°C. Different alphabet notations indicate significance of the effects at type I error (α) of 0.05

## 4. Conclusion

A distinct pattern of colour change between pH 2 to 11 indicated that *Dendrobium* Sonia 'Earsakul' has potential as a pH indicator and natural colourant. Excellent storage stability of DSE crude aqueous extract could be beneficial for manufacturers when preparing and utilizing DSE orchids as a colourant. This application will add value to the cut orchid flower industry and provide another natural colourant alternative to the market. However, further research to verify the types of anthocyanins and optimize the extraction of natural pigments from DSE orchids is needed to effectively evaluate the feasibility of colourant production.

### Conflict of interest

The authors declare no conflict of interest.

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