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Anthocyanin stability studies in *Tibouchina semidecandra* L.

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Abstract

The effects of pH, storage period, temperature, light and dark conditions on the stability of anthocyanins extracted from *Tibouchina semidecandra* flowers of different developmental stages was evaluated. Fully formed but unopened flower bud had the highest amount of total anthocyanin extracted from fresh petals. The anthocyanin contents for all flower developmental stages were stable at pH 0.5–3.0 but the colour of the extracts faded at higher pH values. Degradation percentages of total anthocyanins in the extracts kept at 25 °C were 7–20% lower than that maintained at 31 °C. Extracts stored in darkness at 25 °C maintained their purple colour for 26 days while light exposure reduced it to an average of 10 days. The study shows that suitable storage condition for coloured anthocyanin pigments in extracted form is in acidic conditions in the dark. This implies the potential usage of coloured anthocyanins as natural food colourants and shelf life indicator for acidic foods.

Keywords: Anthocyanin; Colourant; Pigment; Melastomataceae; *Tibouchina semidecandra* L.

1. Introduction

Tibouchina semidecandra L. is a shrub that belongs to the family Melastomataceae. It bears beautiful dark purple flowers throughout the year and grows well in frost-free areas around the world. This plant is used traditionally for both medicinal and food purposes (Zakaria & Ali Mohd, 1994). The pristine purple flower makes it a valuable ornamental plant and a potential source for extraction of natural colourants. To explore the potential of this species as a source of colourant, it is important to understand the conditions that might affect the extraction of the pigments from the plant. Knowledge of the anthocyanin content in relation to floral development is critical for the isolation of the pigments, by indicating the flower development stage at which the coloured pigments are in abundance. In addition, information on the stability of the anthocyanins in extracted form is important to enable further detailed biochemical analyses of the pigments.

Main flower pigments are flavonoids, in particular, anthocyanin, which contribute to the range of red to purple colours found in many flowers, fruits, vegetables and other plant tissues, but are virtually colourless at physiological pHs (Brouillard, 1988; Forkman, 1991; Martin & Gerats, 1993; Mazza & Brouillard, 1990). Anthocyanins are natural, water-soluble, non-toxic pigments. They have not been broadly used in foods and beverages even though they have been reported to be safe in dietary supplements (Bride & Timberlake, 1997). As reported, their susceptibility to colour deterioration during storage and processing limits their application as commercial colourants (Cabrita, Fossen, & Anderson, 2000; Cai, Sun, & Corke, 1998; Mazza & Brouillard, 1990; Tsai, McIntosh, Pearce, Camden, & Jordan, 2002). In fact, the colour stability of anthocyanins depends on a combination of factors, such as the structure and concentration of the anthocyanin, pH, temperature and presence of complexing agents such as phenols and metals (Mazza & Brouillard, 1990). The same anthocyanin may have different

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colours in different plants, depending on the pH of the organelle (Dao, Takeoka, Edwards, Berrios, & De, 1998). They are unlike their synthetic counterparts, which generally obey the Beer–Lambert law and yield stable colours (Bride & Timberlake, 1997).

This study was undertaken to analyze anthocyanins' stability in extracts of *T. semidecandra* flowers of different developmental stages. Parameters affecting the anthocyanin stability in the extract such as pH, temperature, light exposure and storage period were investigated. The overall aim of the study was to identify suitable conditions for the extraction and storage of anthocyanins from this plant species for further detailed biochemical studies.

2. Materials and methods

2.1. Plant materials

T. semidecandra plants were propagated from stem cuttings provided by Dr. Thohirah Lee Abdullah, Faculty of Agriculture, Universiti Putra Malaysia, Selangor, Malaysia. The plants were grown in pots and kept outside the glasshouse under full sun. Fresh petals taken from the matured *T. semidecandra* plants were used throughout the experiments.

2.2. Anthocyanin extraction

To prepare *T. semidecandra* extract, petals at different stages of flower development were collected. Data obtained in this study came from stages S1 (early stage of petal development), S2 (fully-formed petals observed, not yet

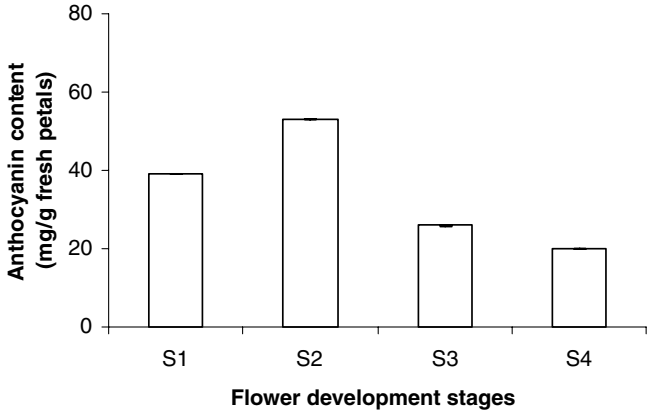


Fig. 2. Anthocyanin content in *T. semidecandra*. Total amount of anthocyanins was calculated based on per gram fresh weight of petals used in the extraction. Values are given as the means ± standard deviations of three independent experiments.

Fig. 1. Flower development stages. S1: early stages of petal development; S2: fully-formed petal observed, unopened; S3: fully-opened flower; S4: wilted petals.

opened), S3 (fully-opened flower), and S4 (petals starting to decay) as shown in Fig. 1. Anthocyanin was extracted by soaking five grams of fresh petals in 50 ml methanol, 1% (v/v) HCl (Harborne, 1984) for an hour at room temperature and in dark condition. The extract was then filtered through Whatmann filter paper for use in subsequent experiments.

2.3. Determination of anthocyanin concentration

Total anthocyanin concentration was determined by measuring the absorbance of the extract at 505 nm using a spectrophotometer (Prim, SECOMAM, France). All measurements were carried out in three replicates and each experiment was repeated three times. In analyzing the effects of light and temperature on anthocyanin stability, the filtered extracts were either exposed directly beneath a 36 W lamp, at a distance of 26 cm, or kept in the dark, at 25 °C or 31 °C. The absorbance of the extract was read daily for 26 days or as otherwise indicated. In analyzing the effect of pH on pigment stability, the extraction was carried

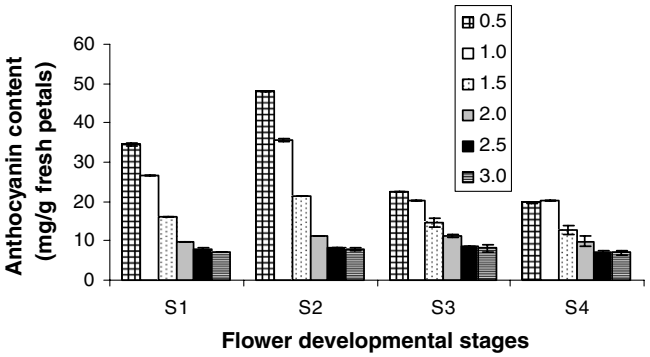


Fig. 3. Anthocyanin concentrations from different flower stages at pH 0.5–3.0. Values plotted are the means of three triplicates ± standard deviations.

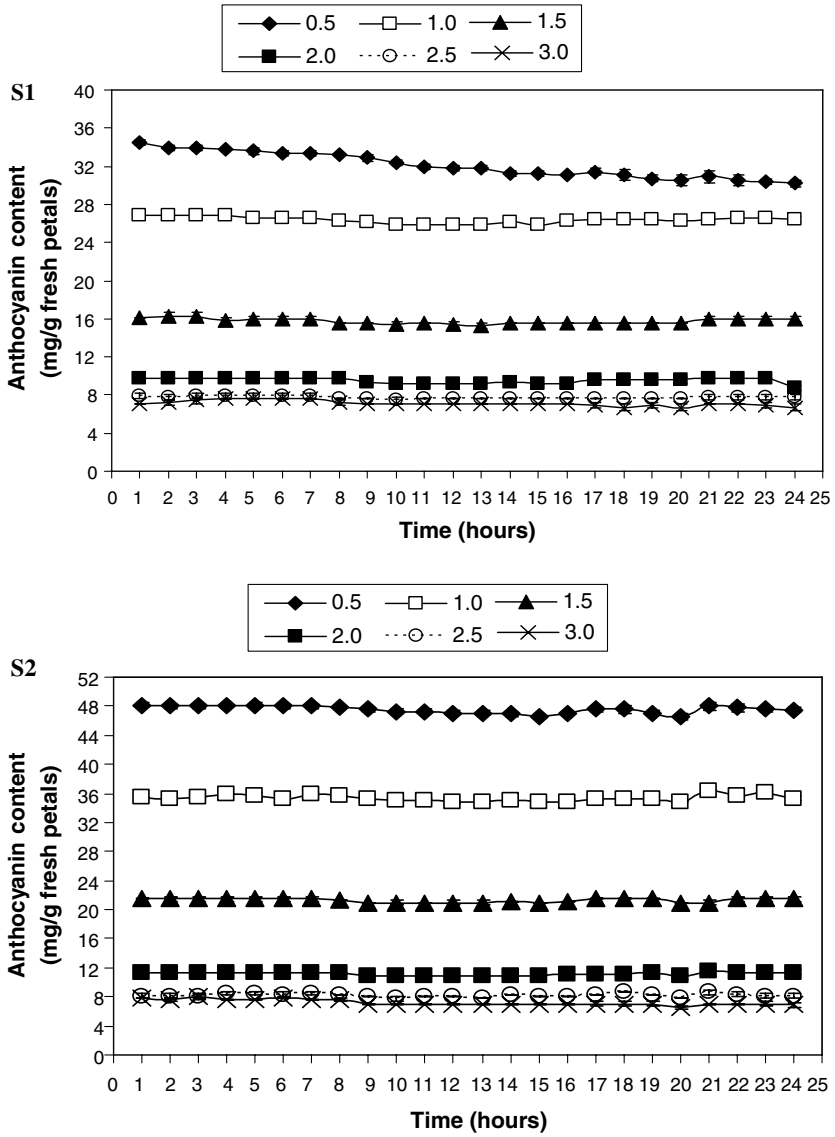
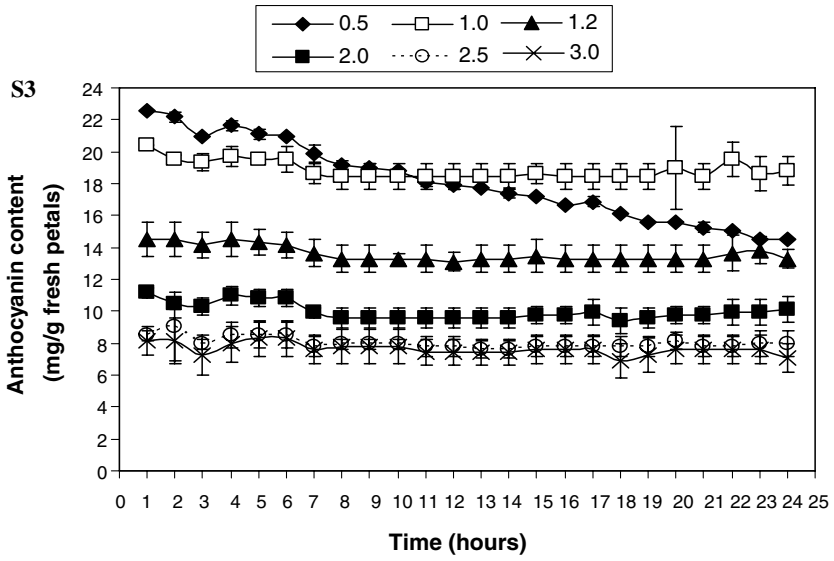


Fig. 4. Anthocyanin concentration in the original extract among the flower developmental stages S1, S2, S3 and S4 at different pH values. Values plotted are the means of three triplicates ± standard deviations.



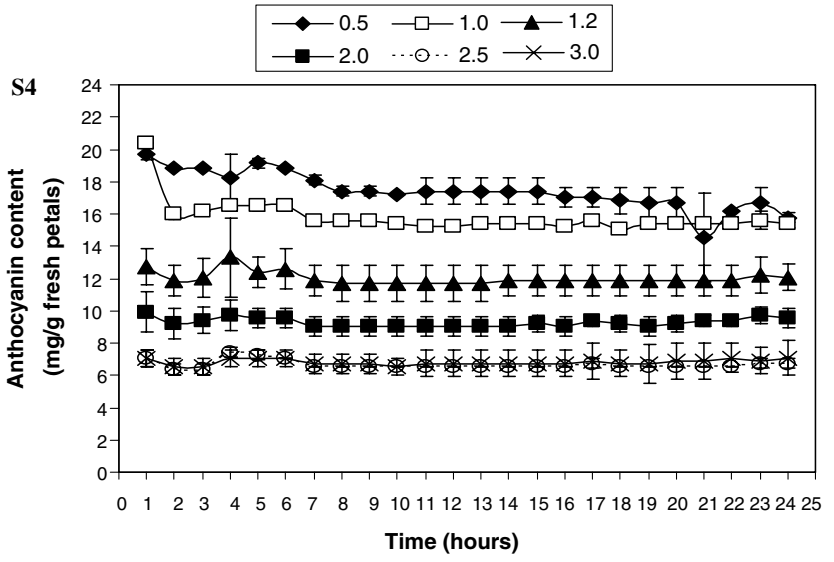


Fig. 4 (*continued*)

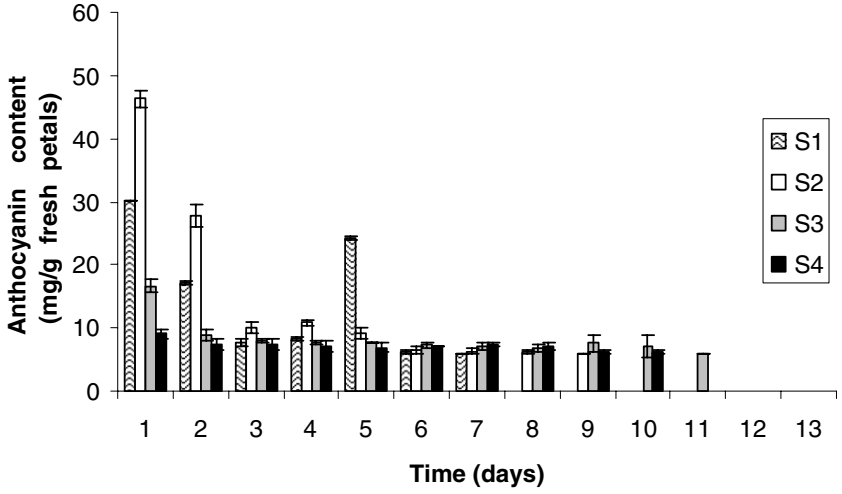


Fig. 5. Anthocyanins content (mg/g fresh petals) in flower development stages S1, S2, S3 and S4 original extracts after direct exposure to light at 25 °C. Values are given as the means ± standard deviations of three independent experiments.

out at different pHs ranging from 0.5 to 3.0, following which absorbance of the filtered extracts was taken hourly for 24 h in three replicates. One-way ANOVA was performed using the SPSS statistical analysis version 12.5. Means for each parameters were compared by Tukey's test at $\alpha = 0.05$.

3. Results

The extract from S2 flower had the highest anthocyanin concentration per gram of fresh petals assayed (51.20 mg/g fresh petals; Fig. 2). This was followed by S1 (39.09 mg/g fresh petals), S3 (25.98 mg/g fresh petals) and S4 (19.88 mg/g fresh petals). Determination of the effect of pH on the efficiency of total anthocyanin extracted was carried out by adjusting the pH of the extracting solution accordingly to values ranging from 0.5 to 3.0 prior to extraction. The results showed that the amount of anthocyanin extracted for all developmental stages varied at different pH values. Maximal amount of anthocyanin was obtained at pH 0.5 for all flower developmental stages (S1 = 34.46 mg/g fresh petals, S2 = 48.16 mg/g fresh petals, S3 = 22.54 mg/g fresh petals, S4 = 19.70 mg/g fresh petals; Fig. 3). At increasing pH, the total amount of anthocyanin obtained decreased for all extracts measured at 505 nm. This effect of pH of the extract was visually confirmed by differences in the colour of the solutions. It was dark purple at pH 0.5 and lighter shades of red at succeeding pHs but turned colourless at pH 3.0. This indicates that the anthocyanin is very susceptible to colour deterioration at different pH values.

Subsequent experiments involved hourly determination of total anthocyanin degradation in the extracts obtained at various pH values. Fig. 4 shows that the amount of anthocyanin obtained at pH values ranging from 0.5 to 3.0 were quite stable for all flower development stages throughout the 24 h observation. There was, however, a slight decline for S3 extract at pH 0.5. We do not know why S3 extract exhibited the slight degradation at that particular pH compared to other pH values.

Exposure of the extracts to light at 25 °C indicated significant changes in the anthocyanin contents for S1, S2, S3 and S4. The amount of anthocyanins for S1, S2 and S3 decreased more than 50% on the third day of light exposure (Fig. 5). This also coincided with the increased fading rate of the extract from dark purple to colourless products. S1 turned colourless on the 8th day, S2 on the 10th day, S3 on the 12th and S4 on the 11th day while none of the extracts maintained in darkness became colourless throughout the experiment.

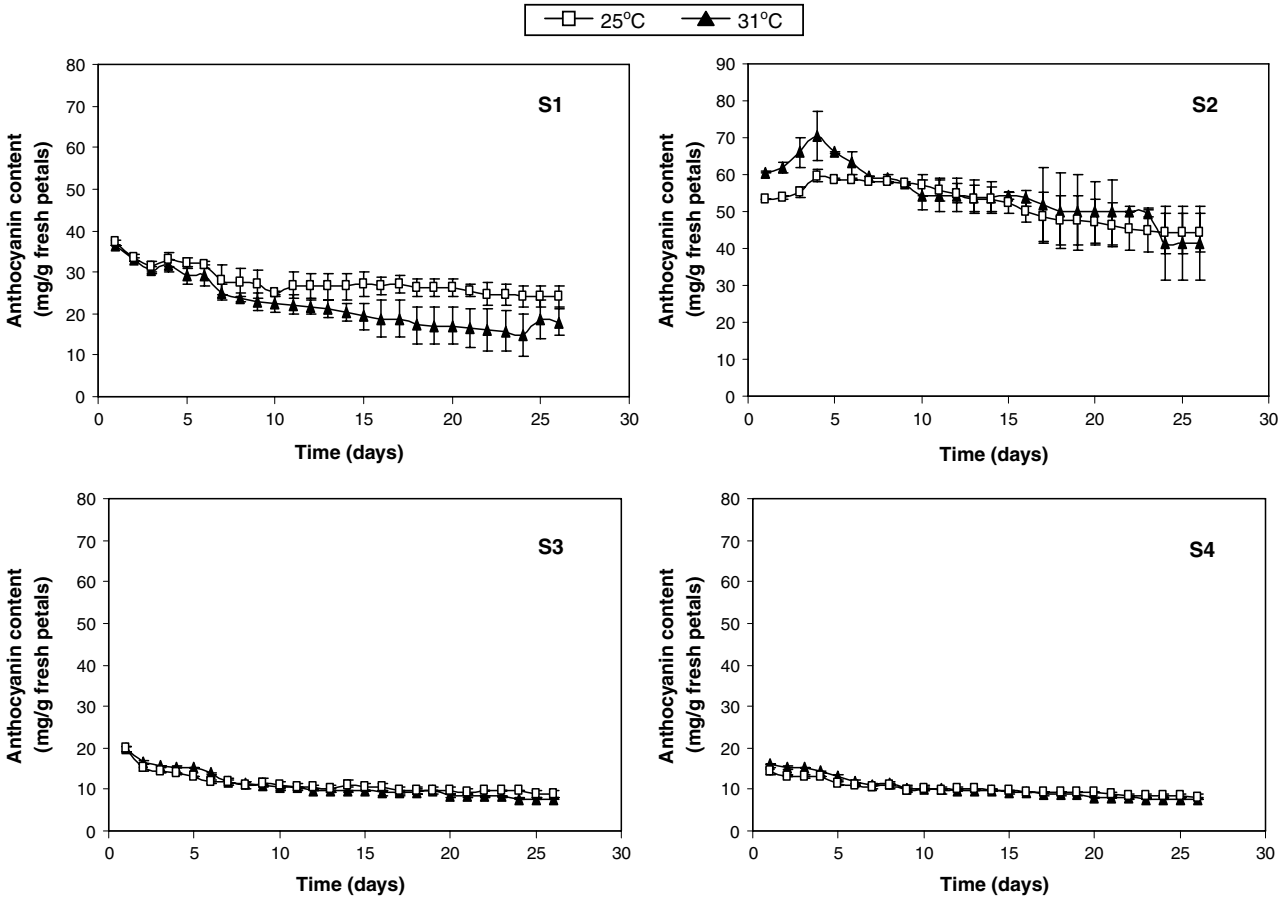


Fig. 6. Anthocyanins content (mg/g fresh petals) in flower development stages S1, S2, S3 and S4 original extracts kept in dark at 31 °C and 25 °C. Values plotted are the means of three triplicates ± standard deviations.

Effects of temperature on anthocyanin content (Fig. 6) were also compared where data were taken at 31 and 25 °C (all extracts stored in dark condition). Even though with a 6 °C difference, the results indicated that at 25 °C the anthocyanin content decrement value was smaller for all flower stages throughout the experiment. After 26 days, the extract kept at 31 °C showed higher total percentage decrement with S1 (48.85%), S2 (11.45%), S3 (53.26%) and S4 (42.99%) compared to extracts kept at 25 °C, which showed S1 (27.83%), S2 (6.65%), S3 (46.96%) and S4 (35.79%), respectively.

Further analysis on the degradation percentage of anthocyanin content on an hourly basis among S1, S2, S3 and S4 extracts stored at 4 °C in darkness showed that the extracts were quite stable (Fig. 7). After 24 h, S2 was shown to have the lowest degradation percentage (6.05%), followed by S1 (12.74%), S3 (14.38%) and S4 (15.92%). The experiment was repeated to study the effect of extended storage days on total anthocyanin content. In extended storage period, the total anthocyanin content was still stable for S1 and S2 (percentage of total anthocyanin degradation after 26 days was less than 10% for both stages) but degraded by 53% and 39% for S3 and S4, respectively, at the end of the observation period (Fig. 8).

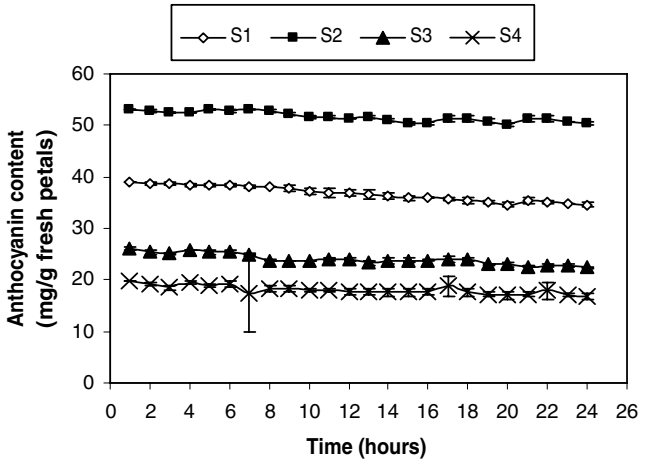


Fig. 7. Anthocyanin concentration in the original extract kept in the dark at 4 °C for 24 h. Values are the means of three triplicates ± standard deviations.

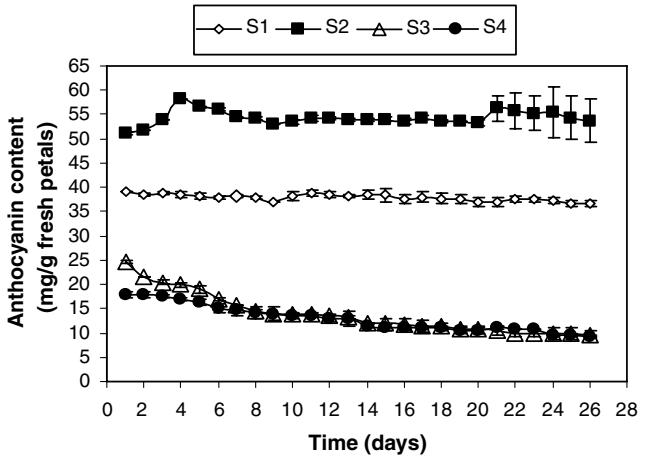


Fig. 8. Anthocyanin concentration in the original extract kept in the dark at 4 °C for 26 days. Values plotted are the means of three triplicates ± standard deviations.

4. Discussion

In this study, it was found that *T. semidecandra* flower at S2 developmental stage exhibits the highest amount of anthocyanin (51.20 mg/g fresh petals). This implies that anthocyanin biosynthesis at this flower developmental stage might be at its maximum level for this species. The rapid increase of anthocyanin content at S2 and the decline after the flowers had matured (S3 and S4) as observed in this study is expected since the colour of the petals at stage S2 is darker than the other flower stages in this species as detected by naked eye. Degradation of anthocyanin as the flowers age has also been observed in *Campanula isophylla* Moretti (Justesen, Andersen, & Brandt, 1997) and *Chrysanthemum* (Stickland, 1972). S2 was also more stable in terms of its rate of anthocyanin degradation (Fig. 7) after harvesting. Our results support the data presented by Cai et al. (1998) that the extent of pigment degradation is dependent on the initial pigment concentration. The high initial anthocyanin concentration correlates with more stable colour, and such higher anthocyanin content in the extract obtained from this S2 stage makes it the choice of flower stage to be used for future experiments.

The pH value of the extraction solution was found to affect the total amount of anthocyanin obtained during the extraction. In this study, pH 0.5 was found to favour the maximal amount of total anthocyanin extracted from each gram of fresh petals for all flower developmental stages. Higher pH values seemed unfavourable for anthocyanin extraction for the species studied here. The colour of the extract obtained was also affected by the pH value in the solution. Colours ranging from dark purple at pH 0.5 to lighter shades of red at higher pH values were obtained. However, the total anthocyanin content in the extracts was quite stable in the pH values (ranging from 0.5 to 3.0) used in the study. Bakowska, Kucharska, and Oszmianski (2003) reported that at pH below 2, the anthocyanin pigment existed as the deep red flavylium ion, and increasing pH hydrated this ion slowly to purple quinonoidal bases. These compounds are labile, and upon nucleophilic attack by water are transformed into the colourless carbinol pseudobase and chalcone pseudobase.

Other factors found to affect pigment stability were temperature and light. Fig. 6 shows that the degradation percentage of total anthocyanin in the extract kept at 25 °C were 7–20% lower than that maintained at 31 °C. This result is consistent with the findings by Shaked-Sachray, Weiss, Reuverin, Nissim-Levi, and Oren-Shamir (2002) and Bolivar and Cisveros-Zevallos (2004) who reported that elevated temperature caused increased pigment

degradation. Like temperature, light also has a degradation effect on anthocyanin (Ochoa, Kesseler, De Michelis, Mugridge, & Chaves, 2001). Throughout the present study, extracts exposed to light showed significant decrement in anthocyanin contents and loss of their characteristic purplish colour (within 8 days as the earliest detected for S1 flower extract). As the temperature was lowered to 4 °C in the dark, the anthocyanin content was quite stable and the purple colour remained throughout the 26 days observation period.

In conclusion, the results showed that stability of extracted anthocyanin obtained from different stages of *T. semidecandra* flowers was affected by temperature, days of storage and light. Extraction at pH 0.5 favours the maximal amount of total anthocyanin obtained. The anthocyanin pigments are stable in the dark at 4 °C for 26 days. The S2 stage flower is at its full-mature state and accumulates the highest anthocyanin concentration. It also exhibits high anthocyanin stability and thus proved to be the choice tissue for future pigments extraction. Data also suggest that anthocyanin extract from *T. semidecandra* can be used as potential colourants in food or as pH indicator in acidic food products. Flower petals of this species are abundantly available as it is a free flowering plant.

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