

A Comparative Analysis of *Clitoria ternatea* Linn. (Butterfly Pea) Flower Extract as Natural Liquid pH Indicator and Natural pH Paper

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(Received: 11 February, 2018; Accepted: 25 April, 2018; Published (web): 10 June, 2018)

ABSTRACT: *Clitoria ternatea* Linn (CT) or Butterfly pea flower is a blue flower edible plant, which is commonly used as food colouring. The objective of this study was to develop a liquid and paper pH indicator from CT extracts. The effectiveness of CT extract as pH indicator was evaluated by titration and product testing result against standard indicator. The stability of CT extracts were also conducted in 7 days storage. Findings from this study shows that CT extract is an effective liquid pH indicator except for weak acid samples. Whereas pH paper made from CT extract is effective as pH paper except for testing pH range from 4 -6. It is concluded that CT extracts can be potentially effective pH indicator.

Key words: *Clitoria ternatea* Linn., butterfly pea, pH, indicator, titration, product testing.

INTRODUCTION

The pH indicator, also known as the acid-base indicator, is a weak acid or weak base which can show colour changes under different acidic and alkaline conditions.¹ A commercially-available pH indicator in a liquid form is commonly used in titration to signal the completion of an acid-base reaction. This indicator may include phenolphthalein, bromothymol blue and methyl orange. A pH indicator also can be in the paper form and used to detect pH changes other than titrimetric analysis. This type of pH indicator includes the litmus paper and universal pH paper.

Commercialized indicators are typically composed of synthetic chemicals and they are produced through chemical treatment/process which can be potentially hazardous and they have the tendency to pollute the environment. In addition, they are not easily available and costly too. Thus, it can be

conclusively stated that there is a demand for a good source of natural indicator and hence it is worth while to do a relevant explorative study.

From literature review, it was noted that more than 50 pigment extracts originated from the plant source had already been investigated for their pH indication properties.²⁻⁶ The common solvent choice for the extraction is ethanol, methanol, water or its combination. Phenolphthalein is the most chosen standard indicator compared to methyl orange.

The natural indicator extracted from the plant source consists of anthocyanin, a subgroup of flavonoids. It is a secondary metabolite that contributes to blue, purple or red colours on fruits, leaves or flowers.⁷ Anthocyanin can react or interact with acids or bases, resulting in changes at the molecular level and hence the exhibition of colour changes (Figure 1 and 2).

For this study, we have used a local, common edible flower petal, *Clitoria ternatea* (CT) or butterfly pea flower as a natural source of indicator.^{3,8-10} CT is a blue flower plant, which is able

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to produce an intense blue colour, which is commonly used as food colouring. Through this study, we have reported the effectiveness of CT flower extract in comparison: liquid versus paper pH indicator; and different acid-base strength combination and stability.

METHODOLOGY

Flower petal *Clitoria ternatea* Linn. A fully bloomed CT flower was purchased from the local market. The flower parts of CT were dried under shade. The green part of the sepal was cut and removed.

Colour pigment extraction. The extraction was performed with slightly modified method from Nyi Mekar Saptarini *et al.* (2015).³ The dried flower and water mixture (1:20) was heated to 35°C with pressing to obtain the maximum yield.¹⁰ After the extracted solution was filtered, testing at sections A, B and C were done accordingly.

A: Development of natural liquid pH indicator from CT. A series of solutions from pH 1-14 was prepared from 0.1N HCl and 0.1N NaOH. Digital pH meter and universal pH strips were used to ascertain the pH values. Two to three drops of CT extract was put in each pH solution to observe the colour change. The colour change was recorded to develop a liquid indicator colour scale for reference as the end point in titration analysis. Four sets of acid-base titration combinations were performed:

1. Strong acid/strong base (SA/SB);
2. Strong acid/weak base (SA/WB);
3. Strong base/weak acid (SB/WA); and
4. Weak acid/weak base (WA/WB).

Phenolphthalein, a liquid indicator, was used as the standard for the titrimetric analysis. T-test was performed to compare the pH indication of liquid and pH paper.

B: Development of natural pH paper from CT. A qualitative filter paper was immersed in the CT extract for 3 - 5 minutes to ensure that the paper pH was fully saturated with the CT extract which was dried at room temperature. The coloured filter paper was then cut into strips as CT pH strips. A colour scale was developed by dipping the CT pH strips

prepared into a series of solution from pH 1 -14 (developed in Section A). The colour changes were observed and recorded as the pH scale. The effectiveness of the pH strips was examined in various product samples. The observed colour changes on the CT pH strips were compared with the colour scale and universal pH paper to confirm the pH of the product tested.

C: UV-Vis spectroscopy and thin layer chromatographic analysis. Water extracts of CT (approximate 0.4 mg/ml) were scanned⁸ through wavelengths 200-700 nm using UV-Vis spectrophotometer (Perkin Elmer, Lambda 35). The UV-Vis data were recorded. The liquid indicator colour scale developed in section A was spotted on the silica gel GF₂₅₄ plate (Merck). TLC was developed in solvent system n-butanol: glacial acetic acid: water (BAW) as recommended for anthocyanin extract.³ The patterns of chromatograms were observed.

Stability test. Stability tests were conducted for the CT extraction day 1 and day 7. The physical changes were observed, recorded and compared. The titration was conducted between the strong acid/strong base and weak acid/weak base combinations.

RESULTS AND DISCUSSION

In the present study, the blue pigment was extracted from the CT flower with the help of distilled water because water is the best medium for an acid-base titrimetric action. Anthocyanin, which is composed of hydroxyl and methoxyl group, is highly soluble in water, hence, provides a promising high yield; the water extract of CT has great thermal stability and so, it has the capability to withstand high temperature.⁸

The anthocyanin content in the blue pigment was confirmed with the qualitative data from UV-Vis spectroscopy and thin layer chromatography. The CT extract showed multiple absorption peak in visible region, 527 nm - 613 nm (Table 1a) and UV region, 223-250 nm (Table 1b). In the visible region, absorption band corresponding to the complementary colour of violet blue was observed. Whereas multiple

peak in UV region was attributed to the multiple transitions from the ground state to the excited state of the molecule due to impure sample.¹¹ In addition, absorption range of 223-250 nm also indicating transition electrons from lone pair and pi electrons to

antibonding sigma and pi bond.¹¹ This is consistent with the dominant group of hydroxyl, methoxy and benzene conjugated group in anthocyanin structures (Figure 1).

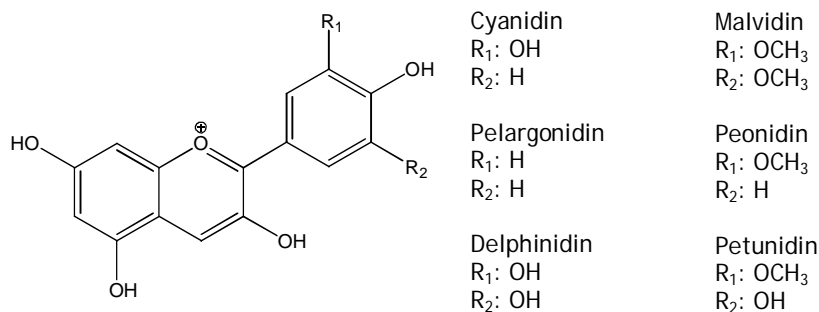


Figure 1. Structure of anthocyanins and its derivatives.

Table 1a. Wavelength (λ) in nm and absorbance for CT aqueous extract by UV-visible spectroscopy (visible range).

λ	527.0	530.0	547.0	557.0	564.0	597.0	609.0	613.0
Abs	0.645	0.000	0.729	0.446	0.541	0.895	0.406	0.532

Table 1b. Wavelength (λ) in nm and absorbance for CT aqueous extract by UV-visible spectroscopy (UV range).

λ	223.50	227.0	231.0	233.98	238.92	242.99
Abs	10.680	10.000	10.00	10.080	10.100	10.000

The TLC chromatogram for the CT extracts in different pH conditions are shown in table 2. Two spots with R_f 0.47 and R_f 0.59 were observed for all the pH conditions. In comparison to the TLC chromatogram for the CT extract (Table 2), three spots were observed, with R_f 0.36, 0.47 and 0.59 respectively. This result indicates that the colour changes in solvent pH 1-14 are contributed by the same chemical constituent as it was from the CT extract. The elution pattern was the same with the previous study.³ The R_f value is closely related to the number of sugars attached to anthocyanin.¹² Sugar is a polar compound and it tends to retain on the TLC plate in such a way that the higher number, the lower the R_f. The R_f of 0.36 was not observed in TLC from pH 1 - 14; this could be due to the fact that the

concentration of anthocyanin was too low for the compound separated to be observed or noted.

The recommended BAW solvent for elution is in the ratio of 5:1:4.¹² In this study, the elution following this ratio was relatively slow, thus, the modification to the ratio of 5:4:1 provides a better elution. The R_f obtained in this study was 0.47 and 0.59, respectively which indicated that that anthocyanin extract was from less polar or less sugar derivative or monoglycoside.¹² The structure confirmation might need further isolation and characterization.

In the liquid indicator pH scale development, the CT extract show different colours under different pH conditions (Fig. 3). The colour varies from red in acidic solution to purplish to blue to green in mildly

alkaline solution to yellow in a very alkaline solution. These changes of colour range are similar to red cabbage purple, corn flowers blue, and poppies red; these changes are characteristic of anthocyanin pigment.² Unlike some commercialized indicator, such as phenolphthalein, only two types of color changes observed (colorless or pink) in acid base

titration. Phenolphthalein undergoes a structural rearrangement as a proton is removed from one of its phenol group as the pH rises (Figure 4). CT extract was able to show multiple colour changes due to more than one proton donation or acceptance group (Figure 2).

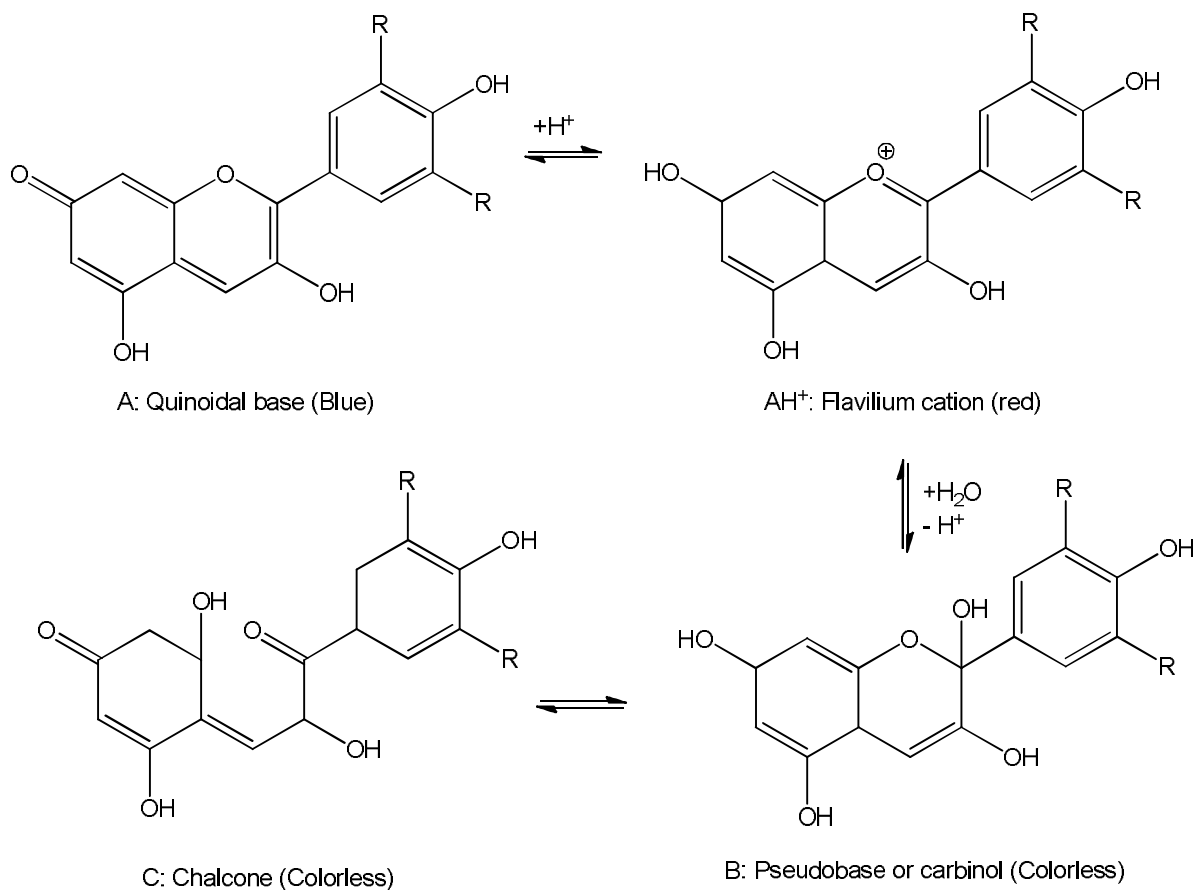


Figure 2. Structural changes of anthocyanins in different pH conditions.

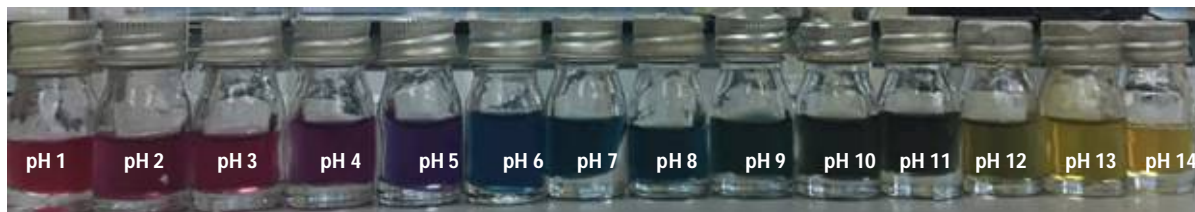


Figure 3. Color of CT extract in different pH condition.

Table 2. R_f value for different pH conditions.

Description	First spot	First spot (R _f)	Second spot (R _f)
3 Drops extract in solution from pH 1 - 14	-	0.47	0.59
Flower extract only	0.36	0.47	0.59

Table 3. Comparison of end point in titration with CT indicator and phenolphthalein.

Description	CT extract	Phenolphthalein	T-test (p-value)	Color change in extract
Strong base vs strong acid (NaOH, 0.1 M vs HCl 0.1 M)	6.27 ± 0.06	6.20 ± 0.17	0.56	Red to blue
Weak acid vs weak base (CH ₃ COOH, 1 M vs NH ₄ O, 1M)	1.90 ± 0.17	2.00 ± 0.17	0.52	Pale green to blue
Strong acid vs weak base (HCl, 0.5M vs NH ₄ OH, 1M)	4.13 ± 0.12	4.47 ± 0.06	0.01*	Red to blue
Weak acid vs strong base (CH ₃ COOH, 2M vs NaOH 0.5 M)	2.47 ± 0.06	2.83 ± 0.35	0.15	Green to blue

P<0.05

Table 4. pH paper from CT extract on product testing.

Product	Color change	CT extract pH paper	Universal pH paper (Standard)
Soup detergent, toothpaste, facial cleanser, body wash, lotion	Blue remained	7	7
Vinegar	Blue to red	2	2
Soda bicarbonate	Blue to green	10	10
Acne care cream	Blue to purple	4	3

Table 5. The physical and chemical stability test result.

Extract	Day 1	Day 7
Color	Blue	Blue
Odor	Herbal odor	Change to unpleasant odor
Texture	Watery	Slimy

Table 6. Comparison of titration result from stability test on day-1 and day-7.

Description	CT Extract	Phenolphthalein	T-test (p-value)	Color change in extract
Strong base vs strong acid (NaOH, 0.1 M vs HCl 0.1 M)	5.20 ± 0.20	6.20 ± 0.17	0.041	Red to blue
Weak acid Vs weak base (CH ₃ COOH, 1 M vs NH ₄ O, 1M)	2.35 ± 0.07	2.00 ± 0.17	0.015	Pale green to blue

*p<0.05

The colour changes are mainly due to the donation and acceptance of a proton from hydroxyl and methoxyl group in the structure of anthocyanin.⁷ In the CT blue extract (as a weak acid), it donates a proton to OH ions in the water, forming anion under

the alkaline solution, forming the base-form, Bb- which is greenish. Hence, the point at which the colour changes from intermediate blue to greenish gives much information on reaching the equivalent point in a neutralization reaction.

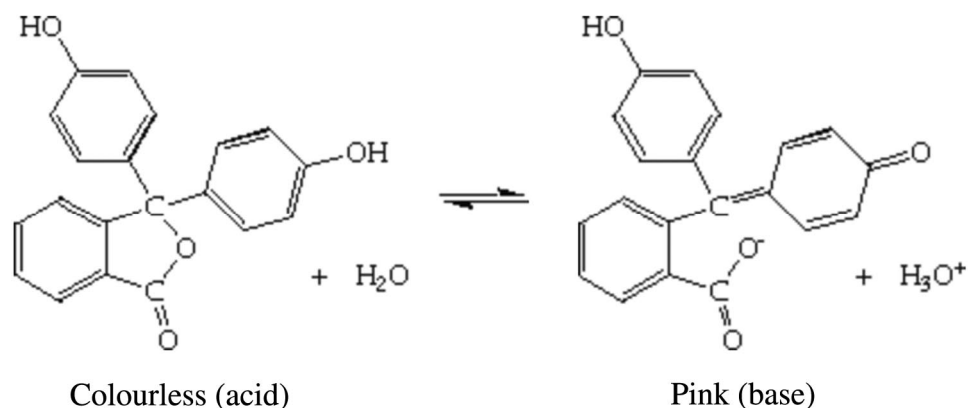


Figure 4. Structural rearrangement of phenolphthalein under pH changes.

From the titration result observed by using phenolphthalein as the standard indicator, all the combinations of titration showed no significant difference ($p < 0.05$) between the end point determined by the CT liquid indicator and phenolphthalein (Table 3). The CT extract showed sharp colour changes to blue upon reaching the end point of titration and this is easy to observe. However, in the strong acid and weak base titration, there is a significant difference between both indicators ($p > 0.05$) (Table 3). In a slightly excess of titrant, the solution turned into red and over to the shoot end point. This is because the strong acid, which fully dissociates even at a low concentration, has higher capacity to react with the weak base. This problem can be overcome by further reducing the molarity of the titrant used.

It has been found that there is a limitation on the CT liquid indicator when titrant is a weak acid. This is because the CT extract appears as purplish colour in a weak acid condition, which is very near to the blue colour as the end point; it is therefore hard to observe the colour change when performing titration. This, thus leads to titration error. So, this study

suggests that the CT extract is a potential acid-base indicator only for an alkaline sample or strong acid sample.

The pH paper made from the CT flower gave an instant colour change when dipped in different product sample (Table 4). However, the colour change was not so obvious in pH ranges 4 – 6, and the violet to blue colour was not easy to differentiate either. As for the product testing, it is likely to be inconvenient because when an opaque semi solid product is tested, the pH paper tends to be covered with the sediments of the product itself, hence the colour changes could not be observed clearly. However, this limitation is also observable in other commercially-available pH paper. Thus, different paper pH materials could be explored to overcome this limitation. We think that the inefficiency of the CT pH paper can be improved by improving the quality of the paper as the CT extract in liquid form could produce a promising outcome.

From the result of the stability test, it was noted that during the 7 days of storage in a refrigerator, the colour remained unchanged (Table 5). However, the odour and texture of the extract dropped in quality. In

terms of the titration test, there is no significant difference between the strong acid/strong base and weak acid/weak base titration (Table 6). The only difference that can be observed is the colour changes are not as sharp as on day 1. This is due to the degradation or oxidation during the storage, causing the changes in the molecular structure of anthocyan in. This explains the displacement of H⁺.

CONCLUSION

Based on this research study, it can safely be concluded that the CT extract can potentially be a good pH indicator as it clearly causes a gradual change in colour over a wide pH range. In fact, the pH of a solution can be approximately identified when a few drops of the CT extract are added to the solution.

The physical condition of the CT extract actually showed light change on day 7. However, this did not affect the end point titration indication. The CT extract is an effective liquid pH indicator as it is able to show rapid, accurate and sharp colour changes when reaching the end point of titration. Furthermore, this liquid pH indicator was found to be good when the sample of analysis was not from any weak acid.

Based on these findings, we recommend that to develop a CT extract pH paper, the qualitative filter paper should be changed to other types of paper which have better absorption capacity. In future study, apart from surveying other paper for making the paper pH indicator, CT can be expanded as indicator in other types of titration such as non-aqueous titration which is commonly used in assay of pharmaceutical products, however, very limited studies were conducted.

Acknowledgements

We are very grateful to laboratory assistant in the Faculty Pharmacy and Health Sciences for their excellent assistance in making this research a great success. A heartfelt appreciation is also due to late Prof. Dr. Mohd Syafiq bin Abdullah who still

remains as a source of strength and inspiration to this researcher.

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