Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(2):275-280

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Application of Butterfly Pea (*Clitoria ternatea* Linn) extract as an indicator of acid-base titration

Nyi Mekar Saptarini[1*], Dadan Suryasaputra[2] and Hera Nurmalia[2]

[1]*Faculty of Pharmacy Padjadjaran University, Jl Raya Bandung Sumedang Km 21 45363, West Java, Indonesia*
[2]*Department of Pharmacy Al-Ghifari University, JlCisarantenKulon 140, West Java, Indonesia*

ABSTRACT

Flowers of butterfly pea (Clitoria ternatea Linn) contain anthocyanins. The color of anthocyaninschange according to the pH solution.The aim of this study is to determine the application of butterfly pea extract as an indicator of acid-base titration. The results showed that the butterfly pea extract has the refraction index from 1.382± 0.25 to 1.390± 0.30, specific gravity from 0.975 ± 0.20 to 0.993± 0.25, maximum wavelength at 572and 614 nm, and discoloration from violet to blue at pH 4, blue to green at pH 9, and green to yellow at pH 12. We concluded that the butterfly peaextract can be applied as an indicator of acid-base titration.

Keywords: *Clitoria ternatea* Linn, flower extract, anthocyanin, acid-base indicator

---

INTRODUCTION

Butterfly pea (*Clitoria ternatea* Linn) are used as folk medicines. The roots and seeds are used as thenerves tonic and laxative. The leaves and roots are used in the treatment of urinogenital disorders, anthelmintic and antidote to animal stings [1].Flowers of butterfly pea contain anthocyanins. Anthocyanins are plant pigment, which are responsible for red violet-blue color in plant flowers [2].There are six major anthocyanins ternatins (A1, A2, B1, B2, D1 and D2) [3]which were characterized as malonylated delphinidin 3,3',5'-triglucosides having 3',5'-side chains with alternating D-glucose and *p*-coumaric acid [4].

The various shades of flower color are due to a very small number of different pigments. These pigments contain the same carbon skeleton, and different only in the nature of the substituent groups [5].The color stability of anthocyanins depend on structure of anthocyanins, pH, temperature, oxygen, light and water activity [6].The color of anthocyanins tends to red in very acidic solution and blue in basic solution [2, 7]. The application of blue anthocyanin-contained flower, likes butterfly pea, has not been optimal yet. This study was aimedto identify the anthocyanins in the flower of butterfly pea and apply its extract as an indicator in acid-base titration based on anthocyanin features.

EXPERIMENTAL SECTION

Materials

Flowers of butterfly pea obtained from Subang, Indonesia. Sodium chloride (NaCl), hydrochloric acid (HCl), potassium chloride (KCl), potassiumhydrogen phthalate, citric acid, sodium citrate, sodium hydroxide (NaOH), potassium dihydrogen phosphate (KH_2PO_4), sodium tetraborate decahydrate ($Na_2B_4O_7$), boric acid (H_3BO_3), sodium bicarbonate ($NaHCO_3$), disodium hydrogen phosphate (Na_2HPO_4), methyl orange, and phenolphtalein were obtained from Merck (Germany).

Instrumentation

The instruments were pH-meter (Boeca), magnetic stirrer, analytical balance (Shimadzu), UV-Vis

spectrophotometer (Shimadzu), and refractometer (Innotech).

Methods

Extraction of Butterfly Pea Flowers

Fresh flowers (100 g) were cutted to 1-2 mm [8]and extracted with 100 mL of distilled water at 25 ºC for 24 hours and at 60 °C for 30 minutes. Each extract was filtered to volumetric flask then rounded up to 100 mL.

Qualitative Analysis

Extracts spotted on silica gel GF254 plate (Merck), then eluted with *n*-buthanol: glacial acetic acid: water (BAW) with ratio 5: 1: 2, 4: 1: 5, 3: 2: 5, and 4: 2: 4. The patterns of chromatograms were observed [2].

Determination of the Maximum Wavelength

Extract (1 mL) was diluted to 5 mL, wavelength is measured using visible spectrophotometer at 500-700 nm [2].

Physical Properties Determination

Organoleptic Test

Organoleptic test consists of determination of the color, smell and taste of the extract [9].

Specific Gravity Determination

The empty pycnometer is weighed, then filled with distilled water and extract, respectively. Extract specific gravity is obtained by dividing the weight of the extract with distilled water at 25ºC [9].

Refractive Index Determination

Refractometer was calibrated with NaCl solution, then 1-3 drops of extract dripped and read the scale. Readable concentration is brix (%), then converted to the refractive index from the correlation table between brix and refractive index [9].

Chemical Properties Determination

The extract (1 mL) was added to 5 mL buffer solution of pH 1 to pH 14, then observed the color change[10].

Butterfly Pea Extract as An Acid-Base Indicator

Weak base-strong acid titration

Solution of 0.1 N $NaHCO_3$ (25 mL) and 10 drops of extract were putted in erlenmeyer and the solution turned green. The mixture was titratedwith standardized 0.1 N HCl. The titration was stopped when the color of solution turned pink. The same procedure was conducted using methyl orange as indicator [11].

Weak acid-strong base titration

Solution of 0.1 N acetic acid (25 mL) and 10 drops of extract were putted in erlenmeyer and the solution turned pink. The mixture was titrated with standardized 0.1 N NaOH. Titration was stopped when the color of solution turned green. The same procedure was conducted using phenolphthalein as indicator[11].

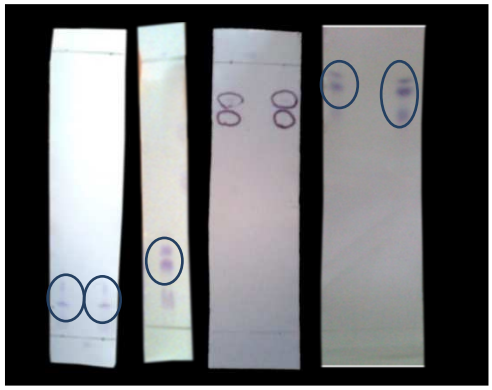
RESULTS AND DISCUSSION

Extraction

Anthocyanin, the flavanoid pigments, are polar. Its soluble in ethanol, methanol, water, and another polar solvents[12].Distilled water is used as a solvent, because the extract will be used as an indicator of acid-base titrations, which performed in water environment. Extraction is done by maceration at room temperature (25 °C) for 24 hours, and by reflux at 60 °C for 30 minutes [13].The aims of this variation is to determine the anthocyanin stability to heat and time of extraction in the water.

Qualitative Analysis

The aims of eluent variation is to identify the variation of chromatogram pattern. Figure 1 showed that more n-buthanol in composition, more slower migration rate of the blue anthocyanin. Whereas, more water in composition, more faster migration rate of the blue anthocyanin (Fig. 1).



(a) (b) (c) (d)

Fig. 1. Chromatogram pattern in BAW in ratio (a) 5:1:2, (b) 4:1:5, (c) 3:2:5, and (d)4:2:4

The Maximum Wavelength Determination

The color of extract of butterfly pea extract was blue. UV-Vis analysis (Fig. 2) gave absorption at the maximum wavelength of 572 nm and 614 nm. Anthocyanin can be distinguished with the other classes by observing the absorption region wavelength of 475-560 nm[14]. The maximum wavelength is different from the literature (475-560 nm). This happens because there are another metabolites were extracted and able to absorb part of the light produced from the spectrophotometer. The colordifference affect the maximum wavelength[13].

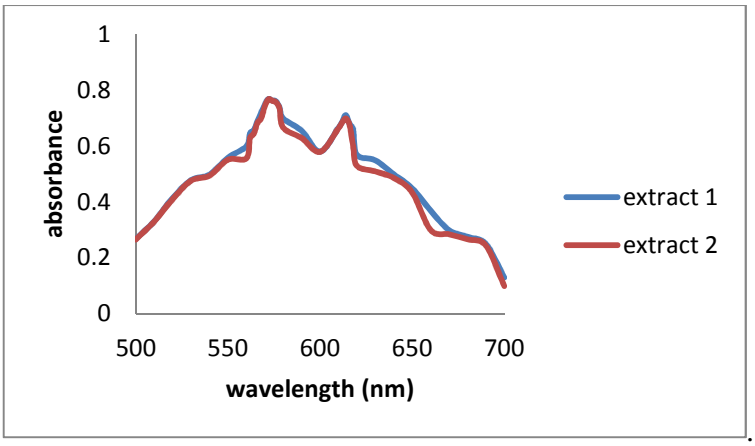


Fig. 2. The maximum wavelength of butterfly pea extract

Physical Properties Determination

Table 1 showed that the color, taste, and smell are not affected by the extraction method, i.e time and temperature. The specific gravity of extract of butterfly pea flower (0.975± 0.20and 0.993± 0.25) is meet the literature, i.e 0.915 to 1.115. This indicates that the extraction method does not affect the specific gravity of the extract. While the refractive index (1.382± 0.25 and 1.390± 0.30) is different from literature (1.450 to 1.470). This happens because the lower extract concentrations with literature, so extract capability to reflect light is lower.

Table 1. Physical Properties of Butterfly Pea Extract

Physical Properties	Extraction method	
	Maceration	Reflux
Color	Bluish purple	Bluish purple
Taste	Tasteless	Tasteless
Smell	Specific	Specific
Specific gravity	0.993 ± 0.25	0.975 ± 0.20
Refractive index	1.390 ± 0.30	1.382 ± 0.25

Chemical Properties Determination

It was observed that butterfly pea extract was blue at pH 7. UV-Vis spectrum (Fig. 2) also gave maximum wavelength at 614 nm. These indicated that butterfly pea extract contained anthocyanin and can be applied as acid-base indicator. Organic compound that can be applied as indicator in titration have characteristic in discoloration in various pH of solution. Discolorationwas happenedthrough equilibrium process of molecule andion

of the indicator. The butterfly pea extract had discolorationfrom violet to blue at pH 4, blue to green at pH 9, and green to yellow at pH 12 (Fig.3).

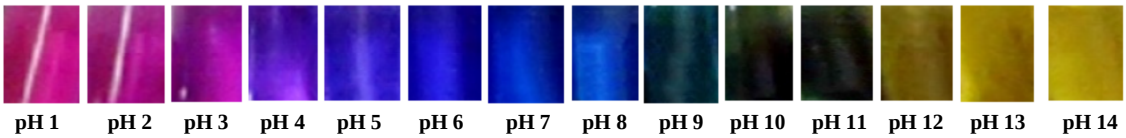


Fig.3.Color change of the butterfly pea extract

The color of anthocyaninsare depend on the acidity of the medium. Anthocyanins in butterfly pea extract had flaviliumcation, which was unstable in the change of pH solution. The change of pH might make the change of anthocyanins structure that led the discoloration(Fig.4). Anthocyanin (structure I), in acidic condition was red. When pH increased (pH < 4), colorless carbinolbase (structure III) would be formed. Tautomer occurred to produce chalcone (structure IV). At pH < 6 the structure changed into anhydrobase (structure II). Extension of conjugation in this structure gave color change to be blue with stronger intensity and maximum wavelength at 610 nm[15].

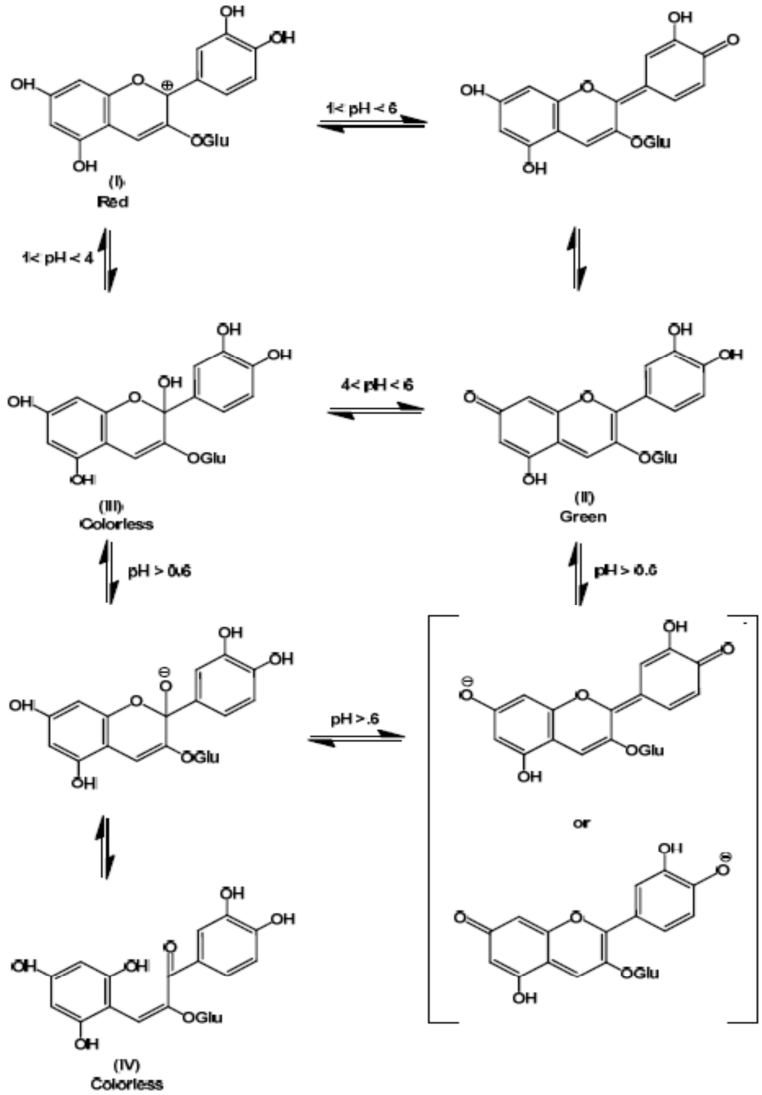


Fig.4. Equilibrium of flaviliumcation of anthocyanin in various pH[15]

Butterfly Pea Extract as AnAcid-Base Indicator

Anthocyanin contained flaviliumcation, which had conjugated double bond, thus led delocalization of positive charge to the whole molecule and gave some structure resonance. The effect of resonance caused the pigment structure can be more stable as flaviliumcation [15]. If nucleophile attacks carbon atom 2 (step a), pseudobasecarbinol(B) will be formed. This species would undergo isomerization into chalcone (c) via water

catalyzed-tautomerization. Then, if a base attacks hydrogen atom of hydroxyl group (step b), quinoid will be formed (Fig. 5). The formation of quinoid would extend the delocalization, thus, gave color change from red in acidic condition to green in basic condition[16, 17].

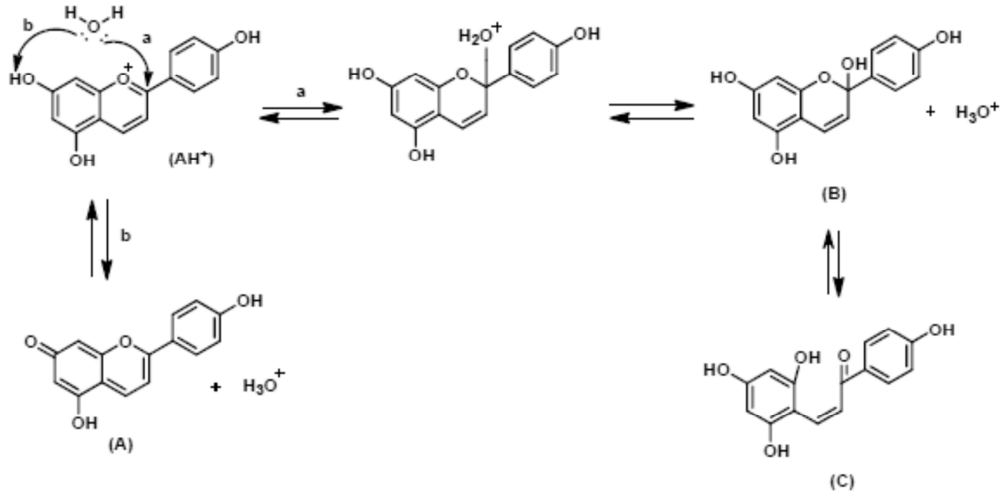


Fig. 5. Reaction of flaviliumcation from apigenidin into quinoid (A), pseudobasecarbinol (B) and chalcone (C)[16, 17]

The discolorationof indicator of butterfly pea extract in weak acid-strong base titration was from pink to green. Whereas, butterfly pea extract gave color change of green to pink in weak base-strong acid titration (Table 2). The pH range of the reference indicator of phenolphtalein and methyl orange are 8.0-9.6 and 4.4-3.1, respectively[11]. We concluded that butterfly pea extract could be applied as indicator in weak base-strong acid titration.

Table 2. Titration condition with butterfly pea extract and the reference indicator

Indicator	Titration condition			
	Weak acid – strong base		Weak base – strong acid	
	0,1 N NaOH (mL)	Color change	0,1 N HCl (mL)	Color change
Extract	25 ± 0.35	Pink - green	25 ± 0.45	Green - pink
Phenolphtalein	25 ± 0.15	Colorless - pink		
Methyl orange	25 ± 0.20		25 ± 0.20	Yellow - red

CONCLUSION

The butterfly peaextract can be applied as an indicator of acid-base titration.

REFERENCES

[1] Anonymous.Indian Medicinal Plants, Vol. 2,Orient Longman,Madras, **1995**.
[2] JB Harborne. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed, Thompson Science, London, **1998**.
[3] N Terahara; N Saito;T Honda;K Toki;Y Osajima.*Phytochemistry*, **1990,** 29, 949-953.
[4] FJ Francis. Miscellaneous colorants, in Natural Food Colorants, GAF Hendry and JD Houghton, Editors. Blackie and Son Ltd., New York,**1992,** 242-247.
[5] IL Finar. Organic Chemistry: Stereochemistry and the Chemistry and of Natural Products, Vol. 2, 5th ed., Longman, London, **1988**.
[6] RL Jackman; JL Smith. Anthocyanins and betalains, in Natural Food Colorants, GAF Hendry and JD Houghton, Editors. Blackie Academic & Professional, Great Britain, **1996.**
[7] K Torskangerpoll; OM Andersen. *J. Food Chem.*, **2005**, 89(3), 427-440.
[8] G Agoes. Teknologi Bahan Alam, PenerbitITB, Bandung, **2007**.
[9] Departemen Kesehatan RI. Parameter Standar Umum Ekstrak Tumbuhan Obat. Dirjen POM, Jakarta, **2000**.
[10] T Supaddit; N Suntorn; P Poungsuk.*African Journal of Biotechnology*, **2012**, 10(82), 19109-19118.
[11] RA Day;AL Underwood. Quantitative Analysis, 6th ed., Prentice Hall of India Pvt. Ltd., New Delhi, **1998**.
[12] Z Anna; KL Bertha;B Sri. *Jurnal Teknologi Kimia Dan Industri*, **2012**, 1(1), 356-365.
[13] N Herlina; P Rahayu, S Notosoedarmo, L Limantara. *Indonesia Journal Chemichal*, **2006**, 6(3), 325-331.
[14] KR Markham. Techniques of flavonoid identification, Academic Press, New York, **1982.**
[15] TJ Mabry; KR Markham; MB Thomas. The Systematic Identification of Flavonoids. Springer Verlag, Berlin,

1970.
[16] R Brouillard; Delaporte. *J. Am. Chem. Soc*., **1977**, 99, 8461-8468.
[17] R Brouillard;GA Iacobucci; JG Sweeny. *J. Am. Chem. Soc*., **1982**. 104(26), 7585-7590.